STUDIES ON THE BIOAVAILABILITY OF DIGOXIN

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CONTENTS

FIGURES

TABLES

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PUBLICATIONS

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SUMMARY

The characteristics of the chemical compounds known as the cardiac glycosides are reviewed, with particular reference to the digitalis group. The clinical use of digitalis is outlined from its origins in folk medicine to the present day. It is pointed out that digitalis is in common use in the treatment of heart disease but the ratio is small between the dosage which produces the desired clinical effect and that which causes toxicity. The pharmacokinetics of the most commonly used cardiac glycoside, digoxin, are summarised. Digoxin was one of the first preparations formulated with the use of the mass-production tabletting process and the features of this type of formulation are described. The concepts of bioavailability of drug formulations and its methods of measurement are discussed and the progress of awareness of its clinical relevance is reviewed.

A description is given of how difficulty in achieving full digitalisation in a patient suffering from paroxysms of atrial fibrillation led to the realisation that absorption of digoxin in that patient was impaired. Changing the brand of digoxin tablet used by this patient to Lanoxin gave more rapid absorption and higher steady state plasma digoxin concentrations. When the patients' original tablets were ground to a fine powder and administered within a capsule there were further increases in absorption rate and steady state concentrations. Similar results were found in a second patient. However, in a further group of 5 patients with low plasma digoxin concentrations or inadequate control of atrial fibrillation no significant rise in plasma digoxin concentrations were seen after

changing to Lanoxin. The plasma digoxin concentrations in a survey of out-patients using Lanoxin and a variety of other brands were lower than those reported a few years earlier in similar patients on equivalent digoxin doses. An explanation of those findings was not apparent until the Burroughs Wellcome Company, manufacturers of Lanoxin, revealed that modications in the production process of Lanoxin were thought to have pronounced effects on bioavailability. In 1972 they announced that a change in production technique a few weeks earlier had doubled the bioavailability of Lanoxin. It became known that an earlier change in 1969 had probably halved Lanoxin bioavailability. The results in the out-patients' survey supported this view of the effect of the 1969 change and suggested that major differences in bioavailability again existed between Lanoxin and many other brands of digoxin tablet.

A comparison of 'newer Lanoxin' made since the most recent production change and 'older Lanoxin' manufactured just before this change confirmed that newer Lanoxin gave higher steady state plasma digoxin concentrations and absorption was more rapid. The mean increase in bioavailability was 70%, but individual patients showed marked variation in their response to a change in formulation, with some showing several-fold increases in digoxin concentration. Newer Lanoxin had a much faster in vitro dissolution rate than older Lanoxin, although both had disintegration rates within the limits stipulated by the British Pharmacopoeia. Measurement of the dissolution rate of 15 other brands of digoxin tablet marketed in the United Kingdom showed a wide distribution of values.

A trial to compare the bioavailability and clinical efficacy of 7 digoxin brands was carried out in 2 groups of cardiac patients,

using steady state plasma digoxin concentration as the measure of bioavailability. This confirmed the difference between older and newer Lanoxin and showed that the bioavailability of the other brands ranged from a bioavailability similar to older Lanoxin to a bioavailability almost equal to the newer Lanoxin. The differences in plasma digoxin concentration were associated with significant differences in the ventricular rate of those patients who had atrial fibrillation. There was a close correlation between bioavailability of these brands and in vitro dissolution rate: there was a correlation coefficient of 0.88 between mean steady state plasma digoxin concentration and the percentage of the dose in solution at 30 minutes.

The profile of the relationship between dissolution rate and bioavailability suggested that there might be an upper limit of dissolution rate beyond which no further increase in digoxin absorption would occur. A study was made of newer Lanoxin and 4 formulations designed to provide very rapid in vitro dissolution. Absorption curves showed that these formulations were all rapidly absorbed. When administered in random order to a group of cardiac out-patients no differences were found in steady state plasma digoxin concentrations. This suggested that a lower limit of dissolution rate of at least 70% in solution at 30 minutes would help to ensure consistent equivalence of bioavailability of digoxin tablets. The British Pharmacopoeia later came to introduce a new requirement that digoxin tablets have a dissolution rate of at least 80% in solution at 1 hour.

To investigate the cause of the differences which had existed in digoxin tablet bioavailability the effect of digoxin particle size was examined. The digoxin powders used for digoxin tablet manufacture were

found to have a log-normal distribution and 50% oversize values from 22 to 29μm. One such powder was mill-ground to produce powders of 12 and 3.7μm sizes. The smaller particle size led to more rapid absorption when these powders were administered within cachets and steady state plasma digoxin concentrations rose as particle size decreased. It was concluded that changes in particle size during tablet manufacture would cause alterations in digoxin bioavailability.

CHAPTER 1

REVIEW

THE CARDIAC GLYCOSIDES

There is in nature a group of substances which share a basic steroid-lactone structure and a particular action on the heart. They are called the cardiac glycosides. Over 300 naturally-occurring or semi-synthetic cardiac glycosides exist (Smith and Haber, 1973). The digitalis plant is the most important source of these substances and the term digitalis is often used for the group as a whole. Digitalis purpurea, the purple foxglove, is native to Britain. It provides three major cardiac glycosides - digitoxin, gitoxin and gitalin (Moe and Mafah, 1970). Digitalis lanata, the white foxglove, is native to Eastern Europe and also contains digitoxin and gitoxin but has digoxin as a third main constituent. The name digitalis was given to the plant by Fuchs (1542) when he provided its first botanical description : it is the Latin equivalent of its German name 'Fingerhut', meaning fingerstall or thimble.

A characteristic action of digitalis on the frog heart was demonstrated by Vulpian (1855). This type of activity and a steroid-lactone structure were found to be possessed by substances in many different plants. Cushny (1925) listed 18 other species of plant known to have cardiac glycosides and Weese (1936) listed 30 sources. These include such disparate origins as lily of the valley, squill, toad skin and arrow poison preparations from Africa.

All of the cardiac glycosides have the same actions on the human heart. They increase the force and velocity of myocardial

contraction and they alter in a characteristic way the electrophysiological state of the heart. The electrophysiological effects are complex and encompass changes in pacemaker automaticity, excitability, membrane responsiveness, conduction velocities and refractory periods, with each effect differing according to the cellular structure involved (Smith and Haber, 1973). A huge amount of experimental evidence has accumulated about the mechanisms by which the cardiac glycosides exert these effects. Schatzman (1953) showed that digitalis had a direct effect on cation transport across the membrane of the erythrocyte. Na^+, K^+ -ATPase was found to be a membrane-bound cation transport enzyme (Skou, 1957) and Repke (1963) postulated that this enzyme system was the receptor for the cardiac glycosides. It is now generally accepted that the electrophysiological effects of all cardiac glycosides are due to changes in membrane potential arising from inhibition of Na^+, K^+ -ATPase and there is considerable but not conclusive evidence that the effect on contraction has the same basis (Hayward and Hamer, 1979; Smith and Braunwald 1980). A hypothesis to fill the gap between a change in Na^+, K^+ -ATPase activity and a change in the actin-myosin contraction process has been developed by Langer (1968) and Akera and Brody (1977). They suggest that an increase in intracellular Na^+ resulting from Na^+, K^+ -ATPase inhibition produces an enhanced influx of calcium ion via the sodium-calcium exchange carrier mechanism. The increase in calcium ion augments contractile element coupling. An explanation of events beyond this step is inhibited by lack of knowledge about the contractile process itself.

The clinical usefulness of the cardiac glycosides is limited by the development of toxicity as dosage increases. Toxicity may be manifest by arrhythmias, nausea and vomiting or disturbance of vision

or mental state: the development of toxicity is due to the electrophysiological alterations within the heart (Mason and Foerster, 1981) and to direct central nervous system effects (Benthe, 1981). In the United Kingdom digitalis toxicity is said to occur in between 13% and 20% of hospital patients receiving a cardiac glycoside (Hurwitz and Wade, 1969; Curruthers, Kelly and McDevitt, 1974). There have now been 34 studies comparing plasma digoxin concentrations in toxic and non-toxic patients, and 12 studies comparing plasma digitoxin concentrations (Smith and Braunwald, 1980). The great majority of these studies show that the cardiac glycoside concentration in the blood was usually 2 - 3 times higher in toxic patients. However there is a degree of overlap between the levels found in toxic and non-toxic patients. These studies re-emphasised the narrow toxic-therapeutic ratio which clinicians have long recognised to exist with the cardiac glycosides.

Chemical analysis of the cardiac glycosides revealed the four molecular characteristics which are necessary for potent cardio activity (Henderson, 1969): they are (1) an unsaturated lactone ring at the C17 position of the steroid nucleus (2) a hydroxyl group at C14 (3) a cis stereochemical arrangement at C13 - 14 (4) one or more sugars at C3.

It is digoxin which has become the most commonly used cardiac glycoside although digitoxin is favoured in French-speaking countries and a number of other plant sources are still used in Russian medicine.

CLINICAL USE OF DIGITALIS

The digitalis plant is one of the most ancient medicines still employed today. The first pharmacopoeia of the Royal College of Physicians in Edinburgh, published in 1699, included digitalis purpurea.

It was among the list of 'simples' i.e. substances of direct plant, animal or mineral origin which were in therapeutic use. At that time the boundary between folk remedies and the medicines used by physicians was indistinct and many of these simples owed their place to their reputation in folklore - for example, cranium hominis violenta morte extincti was also listed among the simples.

In the 17th century digitalis was taken for epilepsy and phthisis or applied externally for wounds, ulcers, scofula and caries (Parkinson 1640, Withering, 1785). Application to the skin was used presumably because of the local irritant action which can be followed by a numbness (Cushny, 1925). However Parkinson (1640) tells us that "there are but few Physitions (sic) in our times that put it to these uses but it is in a manner wholly neglected".

Many of the simples listed in the 1699 Edinburgh Pharmacopoeia were removed from subsequent editions. Digitalis was dropped from the 5th edition of 1756. The foreword explained that the Pharmacopoeia had banished many simples which had been previously included only because of "superstition", "credulity" or "established custom". Also, Salerene (1748) had published his experiments showing severe toxic effects from digitalis in turkeys (this was one of the first animal experiments in pharmacology).

In 1775 William Withering was asked for his opinion on a herbal recipe with which a Shropshire woman, Mrs Hutton, had cured a case of dropsy after the efforts of physicians had failed. There were over 20 ingredients in her recipe but Withering's knowledge of botany allowed him to identify foxglove as the likely active ingredient.

He began to use the foxglove himself and to observe its effects in patients who attended his busy daily clinics. His Treatise of

1785 describes his results in 163 of his own cases and in 46 cases reported to him by colleagues. He found that digitalis could produce a diuresis in cases of dropsy and that it was more potent in these cases than any other medicine then available. He also concluded "that it has a power over the motion of the heart, to a degree yet unobserved in any other medicine, and that this power may be converted to salutary ends".

Withering's care and thoughtfulness as an investigator is shown not only by his shrewd conclusions about the clinical effects of digitalis but by the way in which he sought the best formulation and dosage of the drug. He preferred the use of the leaves, with stalk and midrib removed, collected at the time of flowering in the plant's second year. These leaves he rubbed into powders and they were dispensed as such or made into an elixir or infusion, or, added to soap or gum ammoniac, into a simple pill. However, he cautioned that "the more we multiply the forms of any medicine, the longer we shall be in ascertaining its real dose".

At first he used dosages which were large and frequent and rapidly provoked toxicity. As his experience of the plant grew his concept of the proper method of dosage improved. He noticed that the increased flow of urine preceded evidence of toxicity. Smaller doses produced a period of beneficial effect before any toxicity supervened. He advised that dosing could be stopped for an interval, without losing the benefit, as soon as signs of toxicity appeared. His eminently practical recommendation was:- "Let the medicine therefore be given in the doses and at the intervals mentioned above: let it be continued until it either acts on the kidneys, the stomach, the pulse, or the bowels; let it be stopped upon the first appearance of any of these

effects, and I will maintain that the patient will not suffer from its exhibition, nor the practitioner be disappointed in any reasonable expectation".

It is interesting to look at the doses he suggested. The main emphasis is on a dose of 1 - 3 grains of powdered leaf twice a day i.e. approximately 60 - 180mg B.D. The present recommended daily maintenance dose of prepared digitalis leaf BP is 100 - 200mg per day (Smith, 1973). With Withering's original regime one would expect to find toxicity in about 1 - 3 weeks. However by the year of the publication of his monograph, some 10 years after his first use of digitalis, he became "disposed to believe that the digitalis may be given in small doses, viz 2 - 3 grains per day, so as gradually to remove a dropsy, without any other than mild diuretic effects, and without any interruption to its use until the cure be completed". Withering had reached the concept of avoiding toxicity entirely. However this point was not taken up by his contemporaries who would normally continue digitalisation to the point of toxicity. For some people digitalis developed a reputation of being a dangerous medicine capable of causing sudden death. Dr John Lettsom, then one of London's most emminent physicians, said he had seen eight fatalities during digitalisation. Withering replied that this was the result of excessive dosage used for inappropriate patients (Foy, 1915).

The obvious efficacy of digitalis ensured its popularity. The Edinburgh New Dispensatory (1789) began its section on digitalis with a reiteration of the old uses but added "Digitalis, however, has lately been employed with great success in other diseases. A Treatise has recently been published by Dr Withering ... containing many important and useful observations". William Cullen (1789) in his Treatise on the

Materia Medica advised that Withering's monograph "should be in the hands of every practitioner of physic".

The causes of dropsy were at this time not well understood and although Withering had mentioned the particular effect of digitalis on the heart the plant was initially used as general diuretic. Cullen (1789) wrote: "The powers of this plant as a diuretic are now ascertained by numberless experiments: but upon what sort of operation these powers depend I am at a loss to explain".

Physicians came gradually to realise that digitalis was effective when dropsy was caused by heart disease. They were however puzzled by the apparent paradox of the slowing effect on pulse rate - a 'sedative' effect - and the more positive action of producing diuresis - a 'stimulant' effect. A long and sometimes acrimonious debate developed as to which of these effects was the more important. Ferriar (1799) was the first to emphasise digitalis' effect on the heart but propounded the sedative point of view, with some force, when he wrote "If I am acquainted with an undubitable fact in medical practice, it is the power of digitalis in retarding and weakening the action of the heart and arteries". Bouillard (1835), able to use the new technique of auscultation, described the marked slowing of the heart which digitalis could produce in cases with a rapid irregular pulse, and called digitalis "the opium of the heart".

The controversy was still intense in 1869 when, at a meeting of the Medico-Chirurgical Society of Edinburgh, Bennett clashed with Balfour (1870): Bennett said he was "aware that a modern idea was afloat that digitalis was a stimulant to the heart: this he conceived to be erroneous. He held to the ancient belief that digitalis was a pure sedative to the heart's action". George Balfour, who had carried out animal experiments as well as practicing as a clinician, disagreed and pointed

out that the pulse could sometimes be felt to strengthen before there was any dimination of its frequency. Fothergill (1871), in a lecture on digitalis, stated that digitalis was "..... a drug whose action was unquestionably to produce better, more complete ventricular contraction and in that, and that only, I believe the magic lies".

The arrival of polygraph recordings of atrial and ventricular action demonstrated the nature of atrial fibrillation (Mackenzie, 1905) and showed that digitalis increased atrio-ventricular block. Sir James Mackenzie (1914) concluded that "it is in the treatment of auricular fibrillation that we find the great value of this drug". However he had also been able to record marked improvement from heart failure in a patient with complete atrio-ventricular block in whom digitalisation did not reduce the heart rate (Mackenzie, 1911). Several clinicians, such as Christian (1919) of Boston, reported substantial improvement after digitalisation of patients in sinus rhythm. Nevertheless British views were very influenced by Sir Thomas Lewis who held that the benefit of digitalis lay in extending the diastolic period (McMichael 1972). A crucial stage was reached when Cattell and Gold (1938) showed that cardiac glycosides increased the force and velocity of contraction of isolated papillary muscle. The demonstration of increased contractility in patients awaited the introduction of cardiac catheterisation and cardiac surgery since there was then no adequate animal model for heart failure. McMichael and Sharpey-Shafer (1944) showed that after digitalisation patients with heart failure had a rise in both cardiac output and aortic pressure while filling pressure was reduced. Increased ventricular contraction in the non-failing heart was demonstrated by Braunwald et al., (1961) using strain gauges attached to the ventricular wall at the time of cardiac surgery. Similar

effects were confirmed in the conscious patient at cardiac catheterisation (Mason and Braunwald, 1963, Yankopoulos et al., 1968) and by non-invasive techniques (Weissler et al., 1965).

In addition to its main effects on atrio-ventricular conduction and myocardial contraction, digitalis has other actions on the circulation. It influences vagal and sympathetic tone, peripheral vascular resistance and renal blood flow. It also has non-cardiac effects on the gastro-intestinal tract, adipose tissue, central nervous system and sex hormones (Hayward and Hamer 1979). The important actions however lie in the effects on conduction and contraction - the modern equivalents of the old 'sedative' and 'stimulant' effects - and these form the basis to its current employment in the control of supraventricular arrhythmias and the treatment of heart failure due to myocardial impairment (Smith and Braunwald 1980, Grosse-Brockhoff and Peters 1981). Controversy has not subsided. Some believe there is no long-term benefit from digitalis for patients who are in sinus rhythm and receiving treatment with diuretics (Platt 1975, Lancet 1976). Hamer (1979) reviewed the evidence on this point and concluded that at least some patients with sinus rhythm did derive long-term benefit from digitalisation but if no improvement was discernible, or if digitalisation proved difficult, then a trial period without digitalis was certainly justified. A recent important haemodynamic study (Arnold et al., 1980) showed a substantial and sustained benefit at rest and on exercise in patients with severe myocardial disease who were in sinus rhythm and already receiving diuretic therapy.

DIGOXIN

The chemical content of plants can vary from year to year and from region to region, as demonstrated by the vine. Withering tried

to overcome variation in the content of the active substances in the foxglove by selecting only parts of the plant for use and by gathering them at a specified season. The powder of Prepared Digitalis used today can be standardised by biological assay using frog, cat or pigeon preparations but these methods are imprecise (Stewart, 1981). Attempts to obtain a chemically pure cardiac glycoside began with Hemolle and Quevenue (1844) who extracted a more active principle - amorphous digitaline - from the purple foxglove. However it was only after Nativelle (1869) extracted crystalline digitaline with chloroform that purified glycosides began to be used clinically. Difficulties with the extraction process seem to have been persistent and Dick (1948) detected differences in the control of atrial fibrillation when a variety of commercial digitaline preparations were used by the same group of patients. The purity of digitoxin powder (as the digitaline extract came to be called) remains less demanding than that of digoxin powder (British Pharmacopoeia, 1968).

Digoxin was isolated from digitalis lanata by Smith (1930) who worked in the laboratories of Burroughs Wellcome Ltd. In its crystalline form digoxin is a white, odourless substance with a bitter taste. It is very poorly soluble in water and soluble 1 in 122 in 80% alcohol. The molecular weight is 781. It has a five-membered lactone ring, three sugar molecules attached at the C3 position of the steroid nucleus and differs from digitoxin by having a hydroxyl group at C12 (British Pharmacopoeia, 1968).

Its clinical pharmacology was first investigated by Wayne (1933) who compared oral and intravenous doses and concluded that a solution of digoxin was "fairly completely" absorbed when taken orally. An oral dose of 1.5mg produced much the same effect as 1.0mg intravenously.

The effect of digoxin on atrial fibrillation was seen to be more rapidly dissipated than the effect of digitoxin or digitalis leaf. He suggested that the average dosage of digoxin should be 0.5mg/day.

Similar work was carried out by Rose, Batterman and deGraaf (1942) and by Evans, Dick and Evans (1948).

Gold et al., (1953) carried out further detailed studies on the clinical effects of digoxin in the 1940's. They also observed the effects of oral and intravenous doses in patients who had atrial fibrillation with rapid ventricular rates. From the changes in heart rate they estimated that about two thirds of an oral digoxin dose was absorbed whereas absorption of digitoxin appeared complete. They drew attention to the differences in the doses of oral digoxin needed for digitalisation in their studies and in those reported by the previous workers. They raised the question as to whether this might reflect differences in the digoxin preparations used.

At this time there was no assay method sufficiently sensitive to measure the amounts of digoxin present in the body fluids. The calculation of physiological concentrations of digoxin became possible with the advent of radio-isotopes. After administration of H^3-labelled digoxin, Doherty, Perkins and Mitchell (1961) were able to estimate the amounts of digoxin in plasma, urine and faeces. In a series of studies with radioactive digoxin, Doherty and his colleagues defined the pattern and kinetics of digoxin's absorption, distribution, excretion and metabolism. This work has been summarised by Doherty (1968). His group concluded that after an intravenous dose digoxin was rapidly distributed to the tissues where there was a high degree of protein binding. A large proportion of the dose was excreted into the urine mainly in unchanged form. The rate of excretion was very

dependent on the state of renal function, and digoxin clearance parallelled creatinine clearance and had an inverse linear relationship to blood urea concentration. When renal function was normal, the half-life of elimination was approximately 1½ days but half-life was extended to 7 days in anephric patients. An oral dose of digoxin solution was estimated to be about 80% absorbed. Their data showed that there were considerable differences in the pharmacokinetic profile of different subjects.

The radioactivity of these isotopes prevented them being prepared as normal tablet formulations or being used for long-term administration. During the 1960's, a variety of different assays for plasma concentrations of digoxin became available (Butler, 1972). Two types of assay became popular as they combined adequate sensitivity, accuracy and specificity with relatively rapid and simple technique. One was the radioimmunoassay developed by Smith, Butler and Haber (1969) after Butler and Chen (1967) had succeeded in raising high-affinity antibody to digoxin in rabbits. The other was based on the measurement of inhibition of uptake of rubidium^{86} by erythrocytes. This type of assay was first introduced by Lowenstein (1965), and later improved by Lowenstein and Corrill (1966), Bertler and Redfors (1970), and Gjerdun (1970).

The application of the newer assays has largely confirmed Doherty's conclusions about the main pharmacokinetic parameters. An exception is the pattern of excretion - it is now known that digoxin undergoes a greater degree of metabolism than Doherty and colleagues had thought. Also work in the 1970's, some with the newer assays and some with radioisotopes, has added greater detail to the pharmacokinetic knowledge. After an intravenous bolus dose there is a phase lasting a few minutes in which the digoxin is diluted in the

blood stream. There follows a distribution phase with a half-life of just over 0.5 hours: after a few hours the terminal elimination phase begins, with a half-life of 26 - 44 hours in normal subjects (Greenblatt et al., 1973, Nyberg, Anderson and Bertler, 1974a, Kramer et al., 1974, Koup et al., 1975). This phase is extended to over 100 hours in patients with marked renal impairment (Reuning, Sams and Notari, 1973, Ohnhaus, Spring and Dettli, 1974). In normal subjects between 57% and 80% of the intravenous dose has appeared in the urine in various studies. Renal excretion appears to involve not only filtration at the glomerulus but also tubular secretion and reabsorption (Steiness 1974, Doherty, Ferrell and Towbin 1969).

The digoxin molecule may undergo three types of metabolic change - removal of the sugar molecules, conjugation with glucuronate and sulphate radicals, and saturation of the lactone ring (Reitbrock and Woodcock 1981). These metabolic processes influence the digitalis effect by several mechanisms. Some of the metabolites lose their cardioactivity, although others have similar potency to digoxin. The lipid solubility of the metabolic products is often different to that of digoxin and this change will alter the ability to cross lipid membranes. The elimination rate is also changed: the half-life of digoxigenin monodigitoxoside is half that of digoxin. Particular interest has been paid to the inactive dihydro compounds in which the lactone ring has become saturated. These are found in the urine after an intravenous dose (Lindenbaum et al., 1981a) but occur in much higher amounts after oral doses. Clark and Kalman (1974) found that of 50 patients taking regular digoxin doses 48 excreted a detectable (more than 1ng/ml) amount of dihydrodigoxin, which ranged from 1 to 47% of the total extractable glycoside in the urine. The mean

urine level of dihydrodigoxin was 13%. Greenwood et al., (1975) noted in nine patients that 16.4% (range 12.2% - 19.7%) of the total oral dose was excreted in the urine as dihydrodigoxin. The proportion of total glycoside present as metabolites has tended to be greatest in the faeces, with lesser proportions in the urine and particularly in the plasma. However Watson et al., (1973) when measuring plasma digoxin concentration by gas chromatography detected an abnormal peak in 3 of 150 patients and showed that this peak represented dihydrodigoxin. In one of these cases the plasma dihydrodigoxin concentration was 30% that of digoxin. Lindenbaum et al., (1981b) reviewed the urinary output of reduced digoxin derivatives of 131 normal subjects who had taken part in bioavailability studies. Ten per cent of these subjects showed marked conversion to dihydro forms (greater than 40% of urinary glycoside). They noted that slowly dissolving formulations of digoxin were associated with greater conversion to these metabolites and they suspected that the intestinal bacteria played an important part in the metabolic conversion. The ability of intestinal bacteria to convert digoxin to dihydrodigoxin had been demonstrated earlier in vitro by Herrmann and Repke (1968). Lindenbaum et al., (1981b) subsequently showed that a course of antibiotics caused a marked drop in the excretion of the reduced metabolites. Serum digoxin concentration rose by up to two-fold after antibiotic administration. The influence of the intestinal flora may therefore explain some of the differences in absorption between individuals using the same digoxin formulation.

In vitro incubation has shown that gastric acid can cleave the sugar components from the digoxin molecule (Beermann, Hellstrom and Rosen 1972; Gault et al., 1977). The magnitude of this change is proportional to the pH and to the duration of exposure to the acid.

The extent to which this occurs *in vivo* has not been quantified but the *in vitro* data suggests that the effect is very small when pH is within the normal range.

Within the intestines, digoxin is rapidly absorbed in the upper portions (Beerman et al., 1972). Absorption of doses placed within the colon has been found to be surprisingly extensive, although delayed (Ochs et al., 1975, Anderson et al., 1975).

Gastrointestinal transit time was shown to have an influence on the absorption from slowly dissolving digoxin tablets by Manninen et al., (1973a), who modified intestinal motility with propantheline and metoclopramide. However no significant change occurred when rapidly dissolving tablets were used (Manninen et al., 1973b).

The presence of overt intestinal disease has reduced digoxin absorption in some studies (Heizer, Smith and Goldfinger (1971), Jusko et al., (1974), Brachtel and Gilfrich (1977), Kolibash et al., (1977)) but not in others (Hall and Doherty, (1974), Marcus et al., (1977)).

The total absorption of an oral solution of a cardiac glycoside varies from minimal to complete and is dependent mainly on the degree of lipophilia of the particular glycoside molecule (Lauterbach 1981). Absorption of a digoxin solution is incomplete: different studies have yielded different percentage figures but on average only about three quarters of an oral dose is absorbed (Shaw, 1977). There is variation in extent of absorption of just over two-fold in normal subjects, with each subject tending to be consistent (Johnson, Bye and Lader 1974).

In a fasting subject the peak plasma digoxin concentration occurs at 1 hour: a recent meal reduces the rate of absorption but not its extent (White et al., 1971, Sanchez et al., 1973). Analysis of dietary histories suggested that fat consumption had a very minor

effect on digoxin absorption (Turner et al., 1977). Brown et al., (1978) estimated that a high fibre content meal reduced absorption by 18%.

A large number of reports have accumulated to link reduction of digoxin absorption and co-administration of other drugs (Shaw 1981, Manninen and Nyberg 1981). The drugs involved have been neomycin, sulphasalazine, diphenylhydantoin, para-aminosalicylic acid, various antacids, Kaolin-pectin, cholestyramine and colestipol, and activated charcoal. Spironolactone reduces the clearance of digoxin by a quarter while frusemide causes a mild transient increase. An interaction of greater magnitude has been shown to occur with quinidine. Ejvinsson (1978) first reported than an increase in plasma digoxin concentration was induced by quinidine. Others have confirmed this observation and have shown that the increase, which is on average just under 100%, can induce digitalis toxicity (Manninen et al., 1981). There have been more recent suggestions that verapamil and amiodarone also alter plasma digoxin concentration but the magnitude and significance of these possible interactions has not yet been clarified.

Fortunately digoxin is not strongly bound to plasma proteins and is not vulnerable to displacement by other drugs (Wallace and Whiting, 1974). The low level of hepatic metabolism renders it free from interference by induction of liver enzyme activity.

The picture which has emerged from these more recent studies is one of a complex pattern of absorption, excretion and metabolism, with many factors influencing the steady state plasma digoxin concentration achieved by a particular dose and with considerable variation between individuals. The measurement of the steady state plasma digoxin concentration can be useful in assessing digitalisation as the steady

state level is the accumulated result of the many conflicting influences on digoxin pharmacokinetics in an individual.

TABLETTING

When digoxin began to be marketed by Burrough Wellcome Ltd in the 1930's its attraction lay in the fact that it was a chemically pure substance and doses could be standardised by weight alone.

At the time digoxin was first marketed tablets were beginning to be produced on a mass scale using automated tabletting machines. Burroughs Wellcome themselves designed and constructed their first tabletting equipment and marketed their new digoxin under the brand name 'Tabloid'. Later the brand name was changed to 'Lanoxin'.

A tablet is defined as "a unit dosage form of medication containing one or more drugs to which excipients may have been added and compressed as granules in powder to a definite shape" (Burlinson, 1968). Although the name derives from the circular flat shape, the distinctive characteristic of a modern tablet is its method of creation by compression in a die between punches. The process had been invented by Thomas Brockedon in 1843 but it was not popular initially and medicines continued to be prepared as pills, powders and other traditional formulations. The British Pharmacopoëia of 1885 described only one tablet, glyceryl trinitrate. Even by the edition of 1945 only 35 substances were described in tablet form. However the tablet formulation offered the advantages of accurate unit dosage, ease of handling by patient and pharmacist and gave the economies of large-scale production. It became the dominant formulation for modern drugs and about 60% of prescriptions are now dispensed in tablet form (Burlinson, 1968).

As the unit dosage of digoxin is so small (0.0625 - 0.25mg) the digoxin powder has to be mixed with several hundred times larger volume of excipients prior to tabletting. These excipients are not specified by the pharmacopoeia but are chosen by the manufacturer to supply bulk, lubrication and disintegration qualities to the tablet mixture. These excipients require adequate mixing with the digoxin powder to ensure tablet-to-tablet consistency in digoxin content. The powder is then made into granules to give fast and even flow into the dies of the tabletting machine.

The British Pharmacopoeia of 1968 contained a number of regulations relating to digoxin tablets. A monograph specified the purity of the digoxin powder - not less than 96% of dry weight. The tablets had defined limits for variation in weight. Content was to be checked by assay of the digoxin in a sample of 20 tablets and had to be within 90 - 110% of stated dose. The tablets also had to pass the disintegration rate test. This test was designed to ensure that the tablets had not been excessively compressed and were capable of fragmenting rapidly enough in the gastrointestinal juices to ensure adequate release of the drug for absorption to take place. This was an *in vitro* test in which 6 tablets were to be placed in a chamber which oscillated under water. Within 15 minutes the tablets had to disintegrate and the fragments pass through the wire mesh of the chamber.

The pattern of commercial production of digoxin tablets is large in scale and complexity. In 1972, 245 million digoxin tablets were manufactured for domestic use in the United Kingdom (Committee on Safety of Medicines: personal communication). My own enquiries revealed that within this country digoxin tablets were manufactured by just over

twenty companies. Each company made its tablet in a series of separate batches rather than as a continuous process. A small company might make digoxin tablets only occasionally, sometimes in response to a specific order. A company with many retail outlets might, if its own manufacturing plant was busy, contract out the production of a digoxin batch, although this batch would still be marketed under the companies name.

In addition British companies exported digoxin tablets to many countries and a small quantity were imported. An international company might manufacture digoxin tablets of the same brand name in a number of factories in different countries. Burroughs Wellcome, for example, both exported British-produced Lanoxin tablets and also manufactured their brand, by slightly different techniques, in several centres abroad.

Within the United States, over three dozen companies manufactured digoxin tablets. The Food and Drug Administration reported alarming results relating to the quality control of American digoxin tablet manufacture (Food and Drugs Administration, 1971; Banes, 1971). They had found that 33 of 36 companies produced tablets which failed to meet the weight, content or disintegration rate criteria of their current pharmacopoeia. Inspection of the manufacturing facilities had shown equipment was often excessively worn, an uncritical approach to biopharmaceutics was commonplace, quality control laboratories - although supplied with well-qualified staff - had poor facilities and checks on tablet quality were often omitted.

BIOAVAILABILITY

The term bioavailability was introduced by pharmacists when they became aware that the way in which a drug is formulated can

determine its absorption from the gut. It had long been realised that on some occasions drug absorption could be impaired - it was not rare for a pill to be found intact in the faeces! (Wagner, 1971). However this was attributed to poor preparation by the pharmacist. From the 1930's onwards drug formulation was increasingly in the hands of large pharmaceutical companies who produced a more standardised product, and pharmacists began to study these formulations in a systematic manner. The bioavailability (sometimes called biological availability, physiological availability or systemic availability) of a drug formulation is the pattern of delivery of the drug into the circulation and tissues of the body. It has two components - the rate at which delivery occurs and the total extent to which delivery of the dose takes place. It is, in other words, about how fast and how fully the formulation delivers its dose into the body. The fact that bioavailability covers two components means that it must remain a concept and not a single parameter or measurement. Both components were included because each may independently help to determine the response to the drug. The word bioavailability needed to be invented because no other term covered exactly the same meaning or provided the same emphasis on formulation. Although absorption had a similar connotation for oral formulations it did not cover the possibility that a drug might be totally absorbed from the gastrointestinal tract, but then be metabolised during its first pass through the liver before reaching the general circulation. Also, the term bioavailability could be applied to other methods of administration such as intramuscular injection.

Much of the earlier work on drug formulation characteristics relevant to bioavailability was done <u>in vitro</u> by measuring the rates

at which tablets dissolved and released their drug into solution (Wagner, 1971). The first bioavailability study is said to be that of Oser, Melnich and Hochberg (1945). Although others at an earlier date had observed the absorption of drugs this was the first study with the objective of assessing how formulations of the same substance affected the delivery of the drug into the body. They compared preparations of vitamins and showed that the amounts excreted in the urine after a dose varied between formulations and did not correlate with in vitro disintegration times. The early work was restricted to the few drugs whose concentrations in blood or urine could be measured by assays of sufficient sensitivity. However it soon became necessary to devise ways of quantifying the differences in bioavailability. The problem was that drug concentration changes could normally only be measured in the accessible body fluids such as blood and urine. The solution was found in the compartmental theory, introduced by Teorell (1937) but developed by Riegelman, Loo and Rowland (1968). This conceived the body tissues as simplified into sets of homogenous, discrete compartments. In the most simple version, the one compartment model, the whole body was considered as a single homogenous unit with the blood being representative of all other tissues. In the two-compartment model the blood was considered part of a central compartment into which drug absorption took place and from which all excretion occurred. A second peripheral compartment represented the less highly perfused tissue sites. The drug diffused bidirectionally between these compartments in a manner dependant on the drug concentration in each and on the resistance to diffusion as represented by a rate constant. Models of three, four etc compartments could be

devised but in each case the movement of drug would be defined by a series of rate constants and the total amount of drug entering the system. The compartment theory allowed measurements of drug concentrations in the blood (central compartment) to be used to describe the movement of drug in the body as a whole. The validity of a compartment model could be assessed by following blood concentrations after an intravenous dose of the drug and observing if the curve of decline in drug concentration after injection fitted with the pattern predicted by compartmental analysis. The compartment concept greatly stimulated the study of 'pharmacokinetics' i.e. the study of the time-profile of drug concentration changes in the body. By the early 1970's this work had begun to be summarised and published for a wider audience (Wagner 1971, Notari 1971 and Gibaldi 1971).

In practice most drugs obeyed first-order kinetics (i.e. the amount of drug diffusing out of the compartment was directly proportional to the concentration remaining) and one, two or occasionally three compartment models were sufficient to describe accurately a drug's kinetics. From the mathematical models several practical indices for assessing bioavailability could be derived. After a single dose the area under the time-plot of drug concentration in the blood, when extrapolated to infinity, is directly proportional to the amount absorbed into the circulation. With repeated dosing, drug concentration rises to reach a 'steady-state' at which input balances output. The time to reach steady state can be predicted from the elimination half-life of the drug. The rate of rise is exponential and is 93.75% completed (50 + 25 + 12.5 + 6.25%) after 4 half-lives. At steady state the area under the time profile of drug concentration between doses is a direct

index of the extent of absorption. The drug concentration immediately prior to a dose at steady state is also directly proportional to the extent of absorption, providing absorption is not still occurring. The total amount of drug excreted in the urine after a single dose is also proportional to the amount absorbed, and at steady state the amount excreted in the urine between doses parallels the extent of absorption. The derivation of these indices from the compartmental models was described by Wagner (1971), Notari (1971), Gibaldi (1971) and Greenblatt and Koch-Weser (1975).

None of these indices is expressed in absolute units of absorption and bioavailability must be expressed relative to another formulation. The relative bioavailability of an oral formulation is defined by comparison with a standard liquid preparation. There is no "gold standard" liquid formulation and as absorption can be influenced by the volume and type of solvent and by the position and feeding of the subject the nature of the solution and the circumstances of its administration should always be stated (American Pharmaceutical Association, 1972). Since absorption even from a solution may be incomplete, the absolute bioavailability is found by comparison with an intravenous dose.

These methods of assessing bioavailability assume that the drug is either not metabolised or that metabolism follows a fixed pattern directly proportional to the concentration of unchanged drug. Metabolism of drugs is difficult to study in full and for many medicines this type of knowledge is very incomplete. The pharmacokinetics of metabolites are seldom known. In this respect the digitalis glycosides have been investigated to an unusual extent, particularly for digitoxin

(Storstein, 1981) and digoxin (Reitbrock and Woodcock, 1981). The fact that metabolism can affect measures of bioavailability of digoxin has been shown by the differences found in urinary excretion when digoxin is given intravenously at different infusion rates. Greenblatt et al., (1974) found that a bolus dose of digoxin produced 9% less 6 day cumulative urinary excretion of digoxin, measured by radioimmunoassay, than after infusion of the same dose over 1 hour. Marcus et al., (1976) found that a 3 hour infusion led to 21% higher excretion than a 1 hour infusion rate. These differences have been interpreted as due to higher plasma digoxin concentrations pertaining after rapidly administered doses inducing greater hepatic metabolism (Stoll and Wagner, 1975). A similar phenomenon, but present to a small extent, is likely to occur when oral formulations produce different peak levels of drug in the blood. The radioimmunoassay for digoxin detects the cardio-active metabolites but not the cardio-inactive ones and this "far-sighted" (Larbig and Kochsiek, 1972) characteristic makes the assay generally suitable for bioavailability studies. This however is true only with high-quality antibodies and Rietbrock, Vohringer and Kuhlmann (1977) found one commercial digoxin radioimmunoassay which partially cross-reacted with the cardio-inactive metabolite dihydrodigoxin.

There is therefore a choice of methods available when designing a bioavailability study. The methods chosen will depend in part on the objectives of the study, the facilities available and the nature of the drug. Single dose studies require subjects for whom the drug is not essential. Normal subjects are often sought as they are assumed to have normal drug elimination capabilities and to show less

inter- and intra- subject variability than patients (American Pharmaceutical Association, 1972). Single dose studies have the disadvantage that a very large number of samples has to be assayed to portray accurately the blood drug concentration profile. If a drug has a long elimination half-life, as does digoxin, the collection of blood and urine should be over a period of days and it can be difficult to find subjects able to be available for these periods of time. Studies with measurement of steady state blood or urine drug concentrations have the advantage that they more closely resemble the chronic use of the drug in clinical practice but do involve more exposure to the drug effects (Levy 1974). Steady state measurements have been stated to be the most clinically relevant index of bioavailability (Greenblatt, Smith and Koch-Weser 1976, Keller and Rietbrock 1977). If the subjects are patients it may be possible to record differences in therapeutic affect, but care must be taken to avoid any important deterioration in their health (Koch-Weser, 1974). The immediate pre-dose drug concentration in the blood at steady state has the advantage of simplicity but is sensitive to the precision of the assay used. Accurate measurement of drug excreted in the urine requires very conscientious collection of urine output.

By the start of the 1970's the concept of bioavailability and its methods of measurement were well known to academic pharmacists, although still unfamiliar to most clinicians. In 1961 sufficient evidence (mostly from in vitro experiments) had accumulated to allow Levy and Nelson (1961) to predict that the disintegration rate test of the pharmacopoeia would be inadequate to prevent significant differences in bioavailability of generic formulations of some drugs.

Ten years later Wagner (1971) summarised all the studies to date which had shown variation in bioavailability for drug products of nominal similarity. The majority of these reports involved antibiotics, analgesics or vitamins, for which a modest variation in bioavailability was not of therapeutic importance. However more crucial examples had emerged in clinical practice. The anticoagulant effect of warfarin was found to have been modified by alterations in the formulation of a Canadian brand of the drug (Losinski, 1960). A failure to control diabetes due to formulation effect was found with some brands of tolbutamide (Carter 1963, Carminetsky 1963). In Australia an outbreak of phenytoin toxicity occurred when a pharmaceutical company changed one of the excipient substances, resulting in greater absorption of phenytoin (Tyrer et al., 1970). Azarnoff and Huffman (1976) have more recently summarised the reports showing differences in bioavailability of therapeutic importance.

The limited dissemination of the bioavailability concept by 1970 was reflected by the fact that no pharmaceutical company was, to my knowledge, actively concerned at that time about the bioavailability of its digoxin tablets although digoxin had precisely the characteristics - low water and lipid solubility, incomplete absorption from a solution, and narrow toxic-therapeutic ratio - which Levy and Nelson in 1961 had predicted would lead to bioavailability problems.

CHAPTER 2

PRELIMINARY STUDIES ON THE BIOAVAILABILITY OF DIGOXIN TABLETS

INTRODUCTION

My interest in the bioavailability of digoxin developed as the result of facing a clinical problem in one patient, M.P. This patient had recurrent attacks of atrial fibrillation. When I first encountered M.P. at the out-patient clinic of St. Bartholomew's Hospital in 1971 these attacks had not been controlled by high doses of oral digoxin. The discovery that she had a low concentration of digoxin in her blood led me to investigate her absorption of digoxin.

This case made me examine the literature on digoxin and I found that little had been published about the use of plasma digoxin concentrations to help achieve satisfactory digitalisation, although the radioimmunoassay of digoxin had become available two years earlier. I decided to measure plasma digoxin concentrations in 100 consecutive patients who were attending the cardiac follow-up clinic and who were receiving digoxin treatment. This study had two objectives: (1) to assess the frequency of digoxin toxicity in out-patients and (2) to establish if there were other patients similar to M.P. who had low plasma digoxin concentrations despite an apparently adequate dose. I intended to follow-up the subgroup of patients with low digoxin concentrations and to determine their optimal digoxin dosage requirements, i.e. the daily dose which would give adequate control of their atrial fibrillation or, if they were in sinus rhythm, the dose which produced plasma digoxin concentrations of 1.0 - 2.0ng/ml. This was the range of digoxin concentrations in the blood which Chamberlain et al., (1970) had suggested as the 'therapeutic range'. By the time the survey began

reports had come from the United States (Lindenbaum et al., 1971) and Finland (Manninen et al., 1971) that different brands of digoxin tablet produced differences in plasma digoxin concentration. It was decided to record the brand of tablet used by each of the patients.

The original objectives of the survey of out-patients were superceded when it became evident that in an occasional patient a change in digoxin formulation could produce an important alteration in their state of digitalisation. The results of the out-patient survey and the follow-up studies in individual patients were therefore examined from the point of view of the bioavailability of the tablet brands.

Since an initial objective was to study how digitalisation of patients could be improved the doses of digoxin were often changed in a way which was not ideally suited to a bioavailability study. I have however devoted this chapter to these early studies and shall describe them in some detail as they formed the essential starting point for the future experiments by indicating that there was a bioavailability problem with digoxin tablets.

METHODS

Radioimmunoassay of digoxin

The measurements of the plasma concentration of digoxin in this chapter, and in all subsequent parts of my digoxin bioavailability studies, was by radioimmunoassay.

The radioimmunoassay technique had been conceived by Yalow and Berson (1960) for measurement of poly-peptide hormones. Oliver et al., (1968) applied this technique to the measurement of digitoxin in serum. Butler et al., (1967) succeeded in raising specific, high

affinity antibodies to digoxin in rabbits using digoxin-albumin conjugates. Smith et al., (1969) in Boston used this antibody to develop a radioimmunoassay which was sufficiently sensitive to measure the concentration of digoxin in the plasma of patients. During a clinical attachment from St. Bartholomew's Hospital, Dr Douglas Chamberlain worked in Smith's department at the Massachussets General Hospital and on his return he brought with him supplies of the digoxin antibody which were then used to establish a similar assay procedure in London. In 1970 the antibody produced in Boston was replaced with a digoxin antibody of similar characteristics raised in rabbits in London.

The St. Bartholomew's Hospital assay procedure has been described by Chamberlain et al., (1970). Samples of heparinised blood were centrifuged to obtain plasma which was stored at -10°C until assayed. Each blood sample was assayed in duplicate by taking two 1ml aliquots of plasma. To an aliquot was added 2.2ng of tritiated digoxin (specific activity 3.2Ci per mM : NEN chemicals, Dreieichenhain bei Frankfurt/Main, Germany). Digoxin antibody was then added in a quantity which had been previously found sufficient to bind 55% of the tritiated digoxin in the absence of any unlabelled drug. The mixture was incubated at room temperature for at least 15 minutes. The antigen-antibody complex was separated from the unbound digoxin by absorption of unbound digoxin onto dextran-coated charcoal which was then precipitated by centrifugation. The supernatant was added to 10ml liquid scintillation medium (Bray's solution or NE 250 [Nuclear Enterprises Ltd, Sighthill, Edinburgh]) and heated at 80°C in an oven for 60 minutes to complete denaturation of the antibody and hence release to digoxin from the binding sites. Centrifugation was used to

pack the precipitated protein as firmly as possible to the bottom of the counting phials. The supernatant underwent counting in a Beckman LS100 liquid scintillation counter. Quenching due to plasma constituents was corrected for by use of internal standards. Percentage binding of tritiated digoxin was then calculated. The concentration of digoxin in the sample was obtained from standard curves produced at each assay run by assaying a series of solutions prepared by adding known amounts of crystalline digoxin (British Pharmacopoeia Commission) to pooled normal human plasma.

In this form the assay method had a sensitivity which enabled plasma digoxin concentrations down to 0.25ng/ml, the lower end of the standard curve, to be measured.

Several non-glycoside steroid substances - cholesterol, cortisol, 17B oestradiol, dehydroepiandrosterone, progesterone and testosterone - were assessed and found not to cause interference with the assay.

Two quality control studies of the accuracy and precision of the assay were performed during the period coinciding with the bioavailability studies. In 1971 measured quantities of crystalline digoxin were added to pooled normal human plasma to provide plasma digoxin concentrations of 1, 2 and 3ng/ml. These samples were coded so that they were not identifiable during the assay procedures. The results were:-

1ng/ml sample - mean 0.90ng/ml (30 estimations)
S.D. 0.15

2ng/ml sample - mean 1.90ng/ml (11 estimations)
S.D. 0.15

3ng/ml sample - mean 2.84ng/ml (30 estimations)
S.D. 0.19

During 1972 a similar quality control study was carried out with samples containing 1 - 4ng/ml of digoxin. The results were:-

1.0ng/ml sample - mean 0.85ng/ml (11 estimations)
S.D. 0.05

1.5ng/ml sample - mean 1.46ng/ml (12 estimations)
S.D. 0.05

2.0ng/ml sample - mean 1.92ng/ml (18 estimations)
S.D. 0.10

2.5ng/ml sample - mean 2.43ng/ml (12 estimations)
S.D. 0.15

3.0ng/ml sample - mean 2.92ng/ml (18 estimations)
S.D. 0.15

4.0ng/ml sample - mean 4.02ng/ml (18 estimations)
S.D. 0.22

All of the plasma digoxin assays (including the quality control assays) were performed by M.R. Howard with the exception of the assay results given in Chapter 5 which were performed by Helena Greenwood.

STUDIES IN INDIVIDUAL PATIENTS

Patient M.P.

M.P. was a 50 year old woman who had attacks of paroxysmal atrial fibrillation. She had first attended St. Bartholomew's Hospital in January 1971 when she was admitted via the Emergency Medical Department after presenting with palpitation and breathlessness. She was found to have idiopathic atrial fibrillation and mild pulmonary oedema. She reverted to sinus rhythm after intravenous digitalisation. She was prescribed digoxin 0.25mg/day on discharge.

The next month she was readmitted with a further paroxysm of atrial fibrillation. Practolol 100mg B.D. was added to her regime. She was referred to the Cardiology Clinic for follow-up. In July 1971 she reported having had several more short bursts of palpitation. Her digoxin was increased to 0.75mg/day.

I met M.P. when she attended the clinic in September 1971. She had continued to experience palpitation. Her practolol dose was increased to 200mg B.D. On her return to the clinic two weeks later she reported 5 further attacks. A blood sample was taken for measurement of plasma digoxin concentration to assess her degree of digitalisation. The digoxin concentration was at the lower end of the normal therapeutic range at 1.0ng/ml but I then realised that the blood sample had been taken only a few hours after her last dose. She returned for a further estimation and a sample was collected 12 hours after the preceding digoxin dose : plasma digoxin concentration was reported as being less than 0.5ng/ml. The importance of taking her tablets regularly was emphasised to her - she did claim to have done so previously - and a further sample was taken 2 weeks later. It again contained less than 0.5ng/ml digoxin in the plasma. A further specimen produced the same result.

She was then admitted to the ward on 18/10/71 to investigate the cause of her low digoxin levels. After a period of 6 days without digoxin and following an overnight fast she was given 0.5mg digoxin with 50ml water, using the Boots Ltd brand of digoxin tablets which she had used as an out-patient. Blood samples for measurement of plasma digoxin concentration were taken before the dose and for 8 hours after. The results are shown in Figure 1. The plasma digoxin

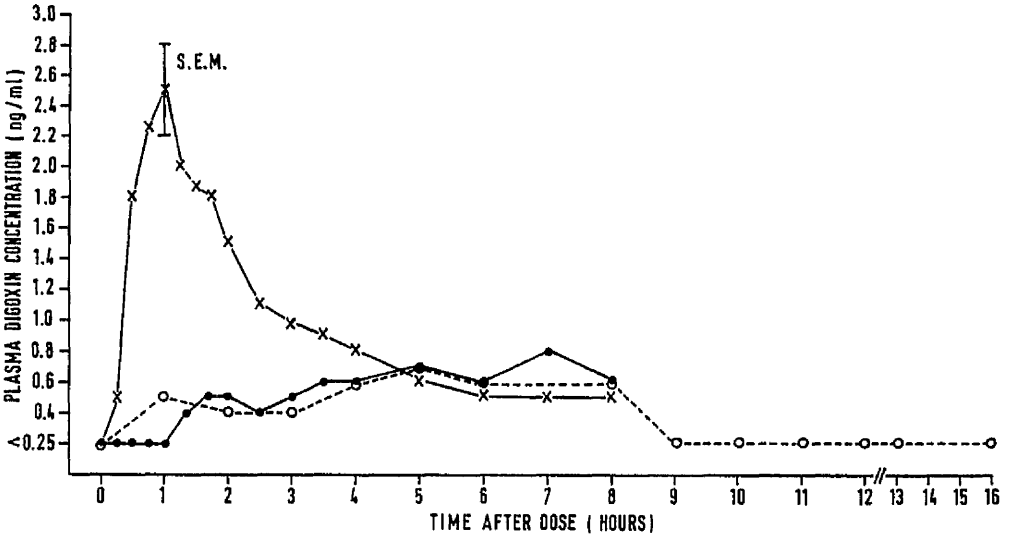

Figure 1 Plasma digoxin concentrations recorded in patient M.P. after 0.5mg doses of Boots Ltd. digoxin tablets on the first occasion (0 - 8 hours, closed circles) and second occasion (0 - 16 hours, open circles). Results obtained in normal subjects by White et al., (1971) are also shown (crosses).

concentrations were considerably lower than those found by Doherty (1961), Marcus et al., (1966) and White et al., (1971) after the same dose given to fasting subjects. The absorption curve recorded by White and colleagues in normal subjects at St. Bartholomew's Hospital is included in Figure 1. They had used Lanoxin tablets. In their study the peak digoxin concentrations had occurred at 1 hour, whereas in M.P.'s absorption curve the peak concentration was at 7 hours. Since this peak level occurred near the end of the sampling period the absorption test was repeated four days later with blood samples taken up to 16 hours after the dose: these results are also shown in Figure 1. No additional peak was seen in this extended absorption curve. An intravenous bolus dose of 0.5mg digoxin was given and frequent blood samples were taken over the initial 3.5 hours. The plasma digoxin concentrations recorded after this intravenous dose were considerably higher than those found after the oral doses: between 15 minutes and 3 hours after the intravenous dose the plasma digoxin concentration fell from 8.0 to 1.9ng/ml. The mean of two samples taken 30 hours after the intravenous dose was 0.9ng/ml. This indicated that her half-life of elimination of digoxin was approximately the same as the normal value of 36 hours found in patients with good renal function (Doherty, 1968).

In view of M.P.'s abnormal digoxin absorption curve it was decided to look for other evidence of intestinal malabsorption. The results relating to this were as follows:-

Haemoglobin level	:	14.7G/100ml	:	normal
Peripheral blood film			:	normal
Serum B_{12}	:	385pg/ml	:	normal
Serum folate	:	2.5ng/ml	:	normal

Red cell folate	:	257ng/ml	:	normal
Serum iron	:	100µg/100ml	:	normal
Iron binding capacity	:	335µg/100ml	:	normal
Total plasma protein	:	7.5G/100ml	:	normal
Albumin	:	4.0G/100ml	:	normal
Plasma calcium	:	9.7mg/100ml	:	normal
Plasma phosphate	:	2.5mg/100ml	:	normal
Alkaline phosphatase	:	57 I.U.	:	normal
3-day faecal fat excretion	:	3.7G	:	normal
Glucose tolerance test	:	fasting 78mg/100ml peak 178mg/100ml at 90 mins		
Xylose tolerance test (50G dose)	:	44mg/100ml at 90 mins	:	normal
Lactose tolerance test	:	rise of blood glucose of 46mg/100ml	:	normal
Barium meal and follow through			:	normal
Histology from biopsy of small intestine (obtained with a Crosby capsule)			:	normal

There was therefore no evidence of malabsorption. To test if she had a very specific absorption defect she was given a 100mg oral dose of hydrocortisone which, like digoxin, has a basic steroid molecular structure. Her plasma cortisol level rose from 11.5µg/100ml before the hydrocortisone dose to reach a peak level of 95µg/100ml at 1 hour after the dose. This rapid rise to a high peak level suggested that her absorption of steroids was not abnormal.

It was concluded at this stage that her impairment of absorption was limited to digoxin. It was decided to increase progressively her

digoxin dosage until a plasma digoxin concentration of between 1 and 2ng/ml was achieved. She was continued on the Boots brand of digoxin and over the subsequent weeks her dosage was increased at 1 - 2 week intervals. The plasma digoxin concentrations found at each dosage level are shown in Figure 2. The blood sample for plasma digoxin concentration was now always taken at 9 - 10 hours after the preceding digoxin dose in view of her prolonged absorption time. Only at remarkably high doses of 1.75mg/day or more did she reach a plasma digoxin concentration above 1ng/ml. During this period she continued to have attacks of atrial fibrillation sufficiently frequently that paroxysms of the arrhythmia were recorded on three visits to the clinic. The ventricular rates which were recorded during these episodes are included in Figure 2. The ventricular rate during the paroxysm tended to be slower as digoxin level increased, although it was rapid even when the plasma digoxin concentration exceeded 1.0ng/ml.

In January 1972 the patient was admitted to St. Bartholomew's Hospital because of chest pain and breathlessness associated with a prolonged attack of atrial fibrillation. It was decided to stop her digoxin treatment and to begin therapy with quinidine (given as Kinidin durules, one 4 times per day) and propranolol (40mg QID). On this regime her attacks of atrial fibrillation were still present but less frequent. By 2nd March 1972 it was felt that digoxin should be added to her regime and that Lanoxin should now be used to test if it gave improved plasma digoxin levels. The plasma digoxin concentrations found after her first 0.5mg dose of Lanoxin are in Figure 3. The Lanoxin tablets gave a slightly greater peak level than had been found after the same dose of Boots digoxin but absorption was much more rapid, with the

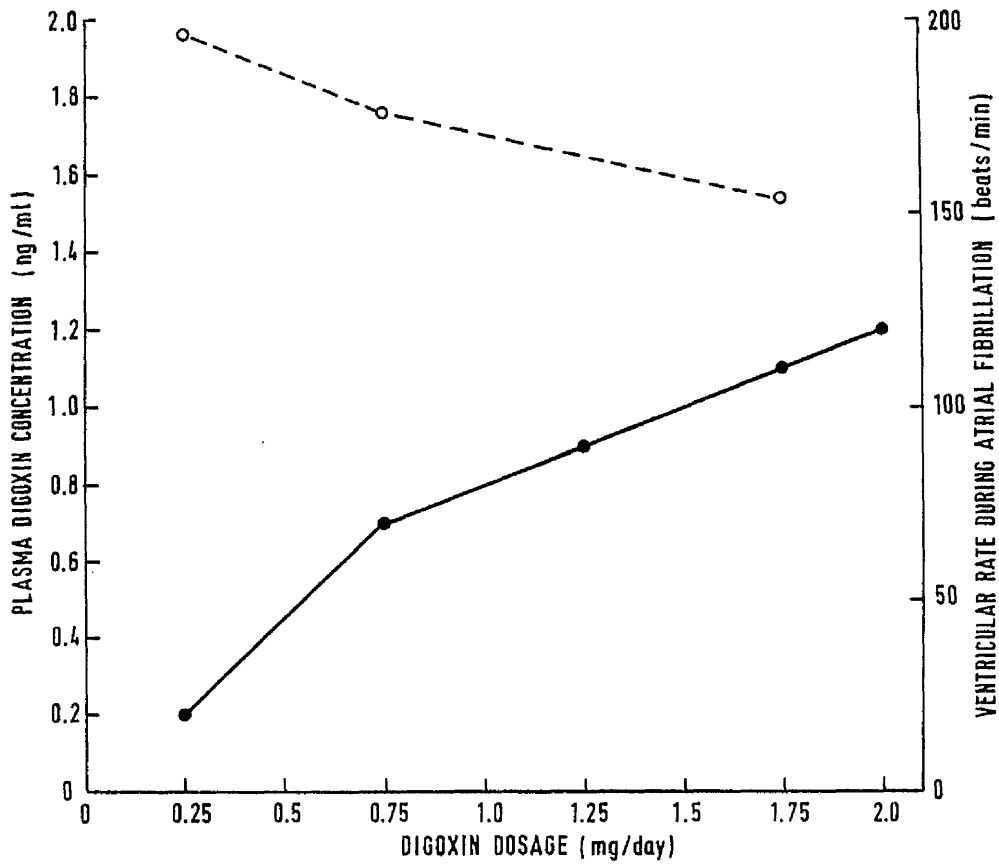

Figure 2 Plasma digoxin concentrations recorded in patient M.P. during maintenance therapy with Boots digoxin tablets (closed circles) and the ventricular rate recorded during attacks of atrial fibrillation (open circles).

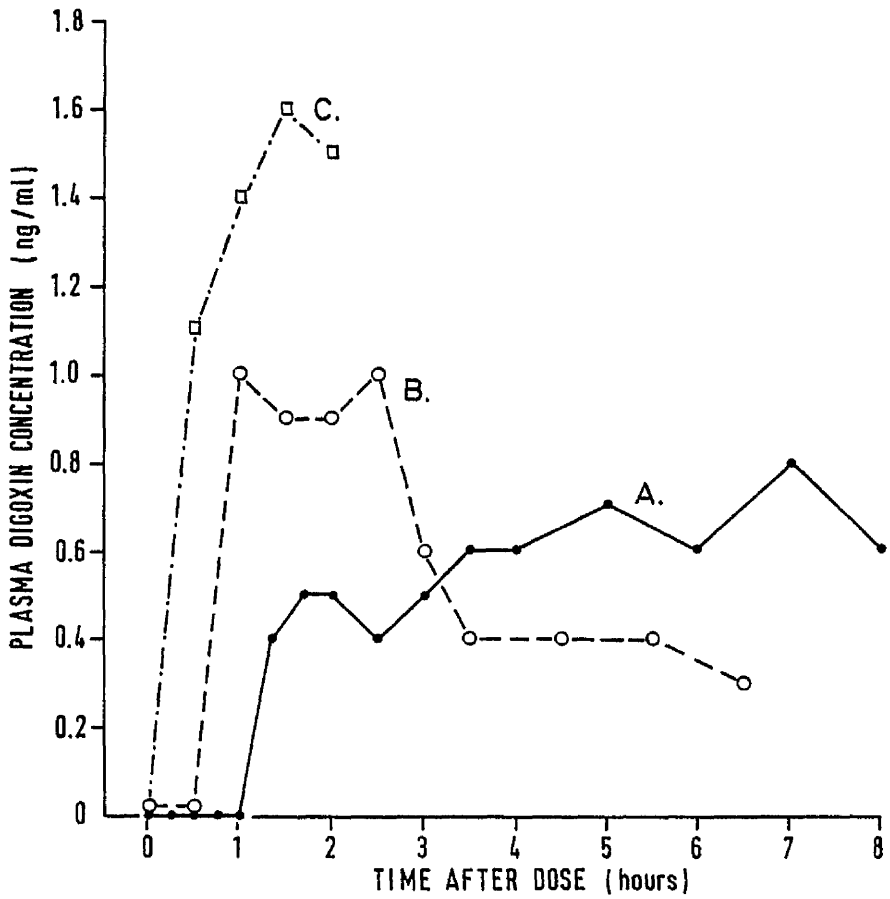

Figure 3 Plasma digoxin concentrations recorded in patient M.P. after 0.5mg doses of Boots digoxin tablets (A), Lanoxin tablets (B) and capsules containing the Boots tablets ground to a fine powder (C).

peak level occurring at 30 minutes after the dose. During maintenance therapy of 0.5mg/day of Lanoxin, blood samples were taken for plasma digoxin estimation at 1, 2, 6 and 8 weeks: each sample was again taken 9 - 10 hours after the digoxin dose. Considerably higher plasma digoxin concentrations of 1.7, 1.3, 1.4 and 2.1ng/ml were recorded on these occasions. An episode of atrial fibrillation had a ventricular rate of 144 beats/min when the digoxin concentration was 1.3ng/ml.

The Boots and Lanoxin tablets used by M.P. were submitted for pharmaceutical analysis. These were carried out for me by Mr. Henry Davis of Harris Pharmaceuticals Ltd., London. Both the Boots brand and Lanoxin (batch 2675) were within the British Pharmacopoeia (1968) standards of weight and size for digoxin tablets. The B.P. disintegration rate test (modified by Mr. Davis to include a smaller size of mesh, gauge 25) gave results within the permitted limit of 15 minutes: Lanoxin - 4 minutes, and Boots brand - 2 minutes. To test if the Boots tablets had been excessively compressed during the tabletting process the pressure required to cause tablet fracture was measured. Five tablets of each brand were tested. The mean pressure to cause collapse or capping of the Lanoxin tablets was 6.2Kg (range 5.8 - 6.4) while the mean pressure to disrupt the Boots digoxin tablet was 1.7Kgm (range 1.1 - 3.1), i.e. 27% of that required to disrupt the Lanoxin tablets. Assay of the digoxin content of single tablets was also carried out using a method based on the British Pharmacopoeia (1968) assay for digoxin content. The content of 10 Lanoxin 0.25mg tablets ranged from 75 to 110% of stated dose and averaged 88%. The Boots tablets gave an average content of 85% of stated dose and ranged from 66 to 117%. The accuracy and reproducability of this single

tablet digoxin assay had not been established by Mr. Davis but it was felt reasonable to conclude that the Boots tablets contained an approximately similar amount of digoxin to that of the Lanoxin.

On 3rd May 1972 these results were discussed with Mr. A.C. Caws of the Quality Control Department of Burroughs Wellcome at Dartford, Kent. Mr. Caws told me that Burroughs Wellcome performed a check of digoxin content on a combined sample of 20 tablets from each production batch of Lanoxin, as required by the British Pharmacopoeia, and he felt that Henry Davis's estimate of 88% of stated content for Lanoxin was too low and probably reflected underestimation by his assay method. Burroughs Wellcome also carried out assays of single tablet content, although this was not required by the Pharmacopoeia, and Mr. Caws undertook to review the results of recent batches. I enquired if it was possible to measure particle size of digoxin within a tablet. Mr. Caws explained that the presence of excipient materials in a digoxin tablet, which constitute 99.8% of the tablet weight, made it extremely difficult to examine particle size of digoxin alone but microscopy of a fragmented tablet gave a rough estimate of digoxin particle size. He subsequently carried microscopic analysis of the digoxin tablets used by M.P. with the following results: Lanoxin - digoxin particle diameters ranged from 3 - 50μm; Boots tablets - particle diameters from 10 - 50μm. It was not possible to differentiate the digoxin particles sufficiently to measure a mean value.

Despite the fact that this pharmaceutical analysis had not revealed any major differences between the Boots and Lanoxin tablets, the faster absorption and higher plasma digoxin concentrations found with Lanoxin indicated that there was some characteristic of these

tablets which affected digoxin absorption. I decided therefore to assess the effect of grinding the Boots tablets into a fine powder and administering this powder within a soft gelatin capsule. The Boots tablets were ground by myself into a very fine powder using a standard ward mortar and pestle. The powder from two 0.25mg tablets were inserted into each capsule. One such capsule was taken by M.P. each morning as replacement of her Lanoxin tablets. The rise in digoxin level recorded during 2 hours of her first 0.5mg digoxin capsule dose are shown in Figure 3: this dose followed previous treatment with Lanoxin 0.5mg/day and the plasma digoxin concentration prior to ingesting the capsule was 2.1ng/ml. Blood samples were also taken after 7 and 10 days of encapsulated digoxin therapy, with the samples taken 9 hours after the dose. Her plasma digoxin concentrations while taking the capsules were 3.5 and 2.3ng/ml. She did not have any symptoms or signs of digoxin toxicity despite these high levels.

She returned to treatment with Lanoxin 0.25mg BD in combination with the Kinidin durules and propranolol.

Patient L.S.

I first met this 61 year old man at the cardiac out-patient clinic on 11/2/72. He had mitral valve disease and chronic atrial fibrillation. He was taking 0.75mg/day of digoxin and used tablets manufactured by Harker-Stagg Ltd. His resting ventricular rate was uncontrolled at 138 beats/min: the plasma digoxin concentration was low at 0.5ng/ml. Two further recordings of plasma digoxin concentration were made at weekly intervals at the same daily dose of this brand and plasma digoxin concentrations of 0.5 and 0.7ng/ml were found. The rise in digoxin concentrations recorded during 6 hours after a 0.5mg dose of

Harker-Stagg digoxin given with 50ml water when fasting are shown in Figure 4. The digoxin level prior to this dose was 0.7ng/ml. The low digoxin concentrations during regular digoxin therapy and the small peak rise after the 0.5mg dose suggested that digoxin absorption was incomplete. In view of the results found in M.P. it was decided to change L.S.'s digoxin brand to Lanoxin. After 2 weeks on 0.5mg/day of Lanoxin he had a plasma digoxin concentration of 1.1ng/ml. His Lanoxin dosage was increased to 0.75mg/day and on blood sampling at subsequent weekly intervals the digoxin levels were 1.0, 0.7 and 0.7ng/ml. His mean ventricular rate, at rest, during treatment with Harker-Stagg tablets was 135/min (S.D. 3) while on Lanoxin the mean rate was 97/min (S.D. 7). This difference in heart rate was, by the unpaired t test, statistically significant ($p < 0.01$). The peak rise in plasma digoxin after a 0.5mg dose of Lanoxin when fasting was 0.9ng/ml (Figure 4) compared with the 0.5ng/ml rise with Harker-Stagg tablets. The Harker-Stagg tablets were analysed in a manner similar to those of patient M.P. The mean tablet content of digoxin was estimated to 83.3% of the stated dose with individual tablets varying from 74% to 104%. The disintegration time was 10 minutes. Pressure to cause tablet fracture was 47% of that required for Lanoxin. Digoxin particle size was found at microscopy to vary from 6 to 150μ. This patient was later given a 0.5mg encapsulated dose of his original tablets after grinding in the mortar and pestle. The peak rise in blood digoxin concentration is included in Figure 4. After 1 and 2 weeks of a daily 0.5mg capsule dose the digoxin concentration in the plasma was 1.7 and 1.3ng/ml respectively.

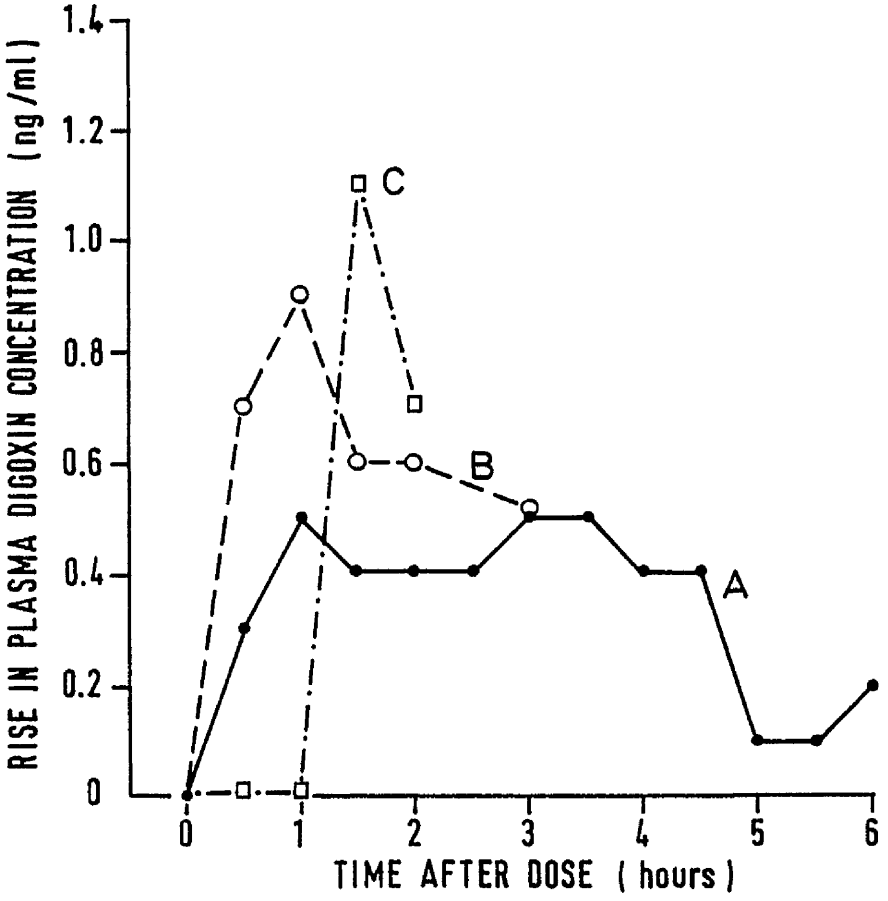

Figure 4 Increase in plasma digoxin concentration recorded in patient L.S. after 0.5mg doses of Harker Stagg digoxin tablets (A), Lanoxin tablets (B), and capsules containing the Harker Stagg tablets ground to a fine powder (C).

OTHER INDIVIDUAL PATIENT STUDIES

Five other out-patients had 2 or more estimations of plasma digoxin concentrations when taking Lanoxin and another brand. They were patients found in the out-patient survey to have unexpectedly low plasma digoxin concentrations or poor control of their atrial fibrillation. Each lived or worked sufficiently near the hospital to be able to attend for several visits. As at this stage I had been interested in determining the daily digoxin dose required to achieve adequate digitalisation in these 'resistant' cases, more than one dosage level was used by each individual. In order to compare in retrospect the bioavailability of their tablets, the plasma digoxin concentrations were adjusted by direct proportion to give concentrations corresponding to a 0.5mg daily dose. The rationale for this adjustment of the measured plasma concentration is that by pharmacokinetic theory the steady state level during regular dosing of a drug with first-order elimination kinetics will increase in direct proportion to the daily dose (Notari 1971, Gibaldi, 1971). This includes the assumptions that absorption from the gastrointestinal tract and any degradation during passage through the liver is unaffected by the dosage level. Since digoxin was known to have a first-order elimination pattern and was not thought to be significantly metabolised during first-pass through the liver (Doherty, 1968) it seemed reasonable to apply this adjustment to plasma digoxin concentrations recorded during use of different daily dosages. Also, it had been shown that steady state digoxin levels did increase in direct proportion to dose in patients who were given stepped increases of digoxin tablet dosage (Redfors, 1972). Although I recognised that this adjustment did not

provide for ideal bioavailability comparisons I felt it was a useful device to allow preliminary comparison of digoxin brands in a situation when it would not have been in the patients best interests to have remained at a fixed dosage of digoxin for prolonged periods of time. Although one could readily see that the plasma digoxin concentrations recorded in patient M.P. as her Boots tablet dosage increased from 0.25 to 2.0mg/day had a different relationship to dosage compared with those found when she took 0.5mg of Lanoxin per day, this adjustment process permitted statistical comparison to be made between the digoxin concentrations obtained with these brands.

The characteristics of these patients are given in Table 1. Their plasma digoxin concentration results are given in Table 2.

The difference in the plasma digoxin concentrations of patient M.P. were statistically significant ($p < 0.01$, unpaired t test) while differences in the other 6 patients did not reach statistical significance (the p value for L.S. was 0.1).

Each of these patients had plasma digoxin concentrations measured for 6 hours after 0.5mg doses of Lanoxin and of the other brand which they had been using when attending the clinic. This dose was given with 50ml water after the patient fasted overnight. The maximal increase in digoxin level during these absorption curves is given in Table 2. There was a weak correlation ($r = 0.46$, $p > 0.05$, < 0.1) between the rise in digoxin concentration and the pre-dose concentration during maintenance therapy with the same brand (Figure 5).

TABLE 1

INDIVIDUAL PATIENT STUDIES : PATIENT CHARACTERISTICS AND MEDICATION

Patient	Age (yr)	Sex	Weight (Kg)	Blood-urea (mg/100ml)	Diagnosis	Medication other than digoxin
M.P.	50	F	82	31	Idiopathic atrial fibrillation	quinidine acid sulphate propranolol
L.S.	62	M	58	31	Rheumatic heart-disease	warfarin frusemide Slow K
H.C.	36	M	63	24	Repaired coarctation of aorta Cardiomyopathy	Navidrex K Slow K
A.M.	58	F	57	27	Hypertensive heart-disease Chronic asthma	frusemide Slow K thyroxine phenindione
D.L.	58	F	73	45	Rheumatic valvar disease Cardiomyopathy	frusemide spironolactone
D.R.	48	F	50	30	Aortic and mitral valve replacements	Navidrex K warfarin
E.P.	60	F	54	34	Aortic and mitral valve replacements	warfarin frusemide Slow K

TABLE 2

INDIVIDUAL PATIENT STUDIES : PLASMA DIGOXIN CONCENTRATIONS DURING MAINTENANCE DIGOXIN THERAPY WITH LANOXIN AND OTHER BRANDS. DIGOXIN CONCENTRATIONS ARE THOSE CORRESPONDING TO A 0.5mg/DAY DOSE

Patient	Brand	Range of dosage used (mg/day)	Number of estimations	Plasma digoxin concentrations adjusted for 0.5mg/day dose		Peak rise in digoxin concentration after 0.5mg dose (ng/ml)
				Mean ± S.D. (ng/ml)	range (ng/ml)	
M.P.	Boots	0.25 - 2.0	5	0.40 ± 0.10	0.3 - 0.5	0.8
	Lanoxin	0.5	4	1.63 ± 0.36	1.3 - 2.1	1.0
L.S.	Harker Stagg	0.75	3	0.43 ± 0.12	0.3 - 0.5	0.5
	Lanoxin	0.5 - 0.75	4	0.70 - 0.28	0.5 - 1.1	0.9
H.C.	Boots	0.75 - 1.25	3	0.66 ± 0.12	0.6 - 0.8	0.4
	Lanoxin	0.5	3	0.76 ± 0.21	0.6 - 1.0	0.6
A.M.	Unknown	0.5 - 0.75	4	0.68 ± 0.21	0.5 - 0.9	0.6
	Lanoxin	0.375 - 0.5	3	0.83 ± 0.32	0.6 - 1.2	0.9
D.L.	Boots	0.5	2	0.90 ± 0.00	0.9 - 0.9	1.1
	Lanoxin	0.5 - 0.625	5	1.06 ± 0.13	0.9 - 1.2	1.2
D.R.	Boots	0.5 - 0.75	3	1.07 ± 0.15	0.9 - 1.2	1.1
	Lanoxin	0.5	2	1.20 ± 0.14	1.1 - 1.3	0.6
E.P.	Boots	0.5 - 0.625	3	1.40 ± 0.10	1.4 - 1.5	0.7
	Lanoxin	0.5 - 0.625	2	1.25 ± 0.21	1.1 - 1.4	1.2

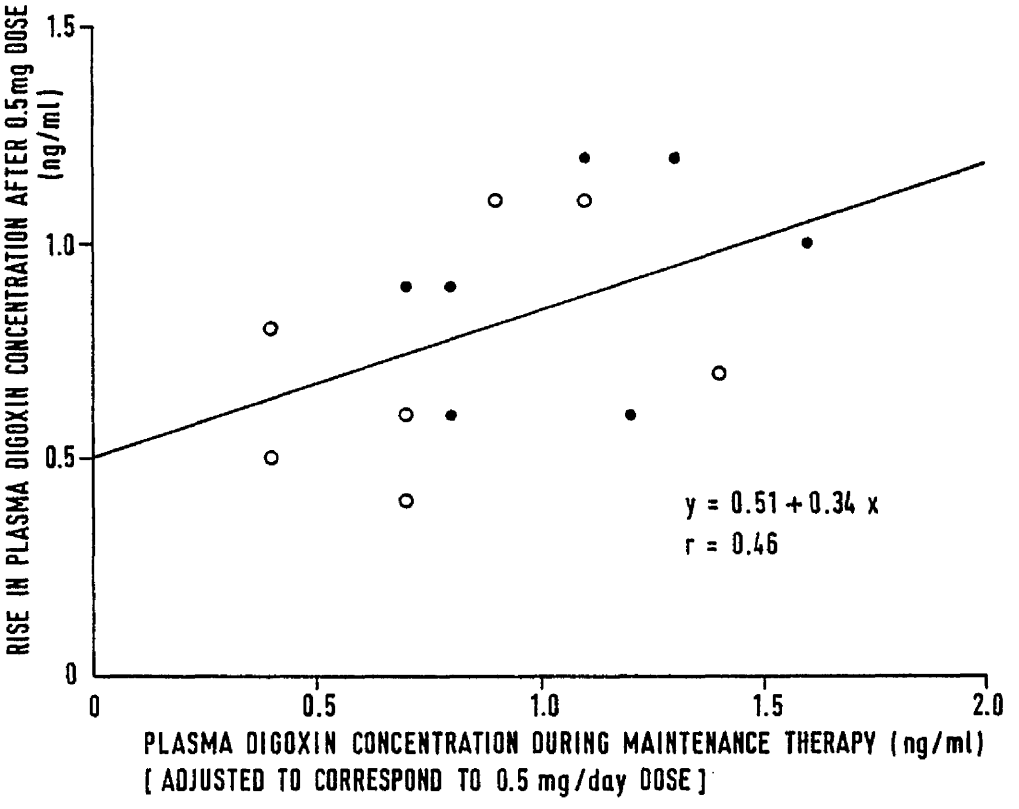

Figure 5 Relationship between peak rise in plasma digoxin concentration after 0.5mg oral digoxin dose and pre-dose plasma digoxin concentration during maintenance therapy with the same brand of tablets. Closed circles = Lanoxin, open circles = other brands. Digoxin concentrations for maintenance therapy have been adjusted to correspond to a 0.5mg/day dose (see text).

SURVEY OF OUT-PATIENTS

The casenotes of all patients attending Dr. John Hamer's afternoon follow-up out-patient clinic during the period 12th February - 12th June 1972 (excluding a 2 week vacation interval) were reviewed. Those patients on digoxin treatment were interviewed. The age, sex, clinical diagnosis and drug treatment were recorded and their weight and height were measured. Each patient was questioned about their digoxin dosage and the time interval since their last dose. A 20ml sample of venous blood was taken for estimation of urea and electrolytes, thyroid function tests and plasma digoxin concentration: the sample was timed to be 6 - 10 hours after their last digoxin dose. A 12 lead electrocardiogram and 60 second V_1 rhythm strip were recorded.

Patients who had not brought their tablets to the clinic were asked to send an example of their current digoxin tabletand the name and address of the pharmacist who had dispensed their prescription. Lanoxin tablets could be identified by their characteristic markings. When the tablet was seen to be other than Lanoxin the pharmacist was contacted by telephone and asked for the name of the manufacturer whose digoxin brand he used. In some instances the retail pharmacist had obtained his supplies from a wholesale chemist and was unaware of the manufacturer. In this circumstance the wholesale chemist was contacted. The brand of unmarked digoxin tablet was able to be identified in all but 11 instances: in these the retail chemist had recently used more than one brand (7 cases), the patient was unable to give details of the dispensing pharmacist (2 cases), or the patient was using a bottle of digoxin tablets containing a mixture from more than one prescription (2 cases).

The results of the survey were analysed after 76 patients had been interviewed. Forty-three had been using Lanoxin and 33 used other brands. Seven brands other than Lanoxin were identified. As no single brand was used by more than a small number of patients, these 33 patients were combined into an 'other brands' group. This predominance of Lanoxin corresponded with the national sales pattern. During 1971 the market shares of digoxin tablet sales were: Lanoxin 61%, Boots brand 15%, Kerfoot brand 7%, Evans Medical brand 4%, Cox brand 3%, others 10%. (C. Oakley, Department of Health and Social Security: personal communication, 1972).

The patient characteristics and plasma digoxin concentrations found at each daily dosage level are given in Table 3. The patients using Lanoxin and other brands were similar in terms of age, weight and blood urea level. No statistically significant differences (unpaired t test) were found in plasma digoxin concentrations of those taking Lanoxin and those using other brands except for the smallest group receiving 0.625 - 0.75mg/day of digoxin ($p < 0.05$).

DISCUSSION

Three conclusions emerged from these preliminary studies of digoxin bioavailability: (1) some patients (M.P. and L.S.) showed an improvement in their state of digitalisation after a change in their brand of digoxin tablet (2) there was, overall, no major difference in the bioavailability of Lanoxin and other brands (3) grinding some digoxin tablets to a fine powder increased their absorption.

TABLE 3

PATIENT CHARACTERISTICS AND PLASMA DIGOXIN CONCENTRATIONS RECORDED IN SURVEY OF OUT-PATIENTS

Digoxin dosage (mg/day)		Lanoxin (Means ± S.D.)	Other brands (Means ± S.D.)
0.125 - 0.25	Number of patients	16	16
	Weight (Kg)	56.7 ± 12.2	65.7 ± 9.8
	Blood urea (mg/100ml)	44 ± 13	38 ± 13
	Plasma digoxin concentration (ng/ml)	0.59 ± 0.30	0.58 ± 0.25
0.375 - 0.5	Number of patients	20	14
	Weight (Kg)	61.0 ± 12.8	58.6 ± 8.5
	Blood urea (mg/100ml)	40 ± 16	38 ± 7
	Plasma digoxin concentration (ng/ml)	0.94 ± 0.37	0.90 ± 0.27
0.625 - 0.75	Number of patients	7	3
	Weight (Kg)	57.9 ± 10.7	66.8 ± 11.2
	Blood urea (mg/100ml)	30 ± 5	27 ± 8
	Plasma digoxin concentration (ng/ml)	1.24 ± 0.29	0.77 ± 0.23

Patient M.P. had very low plasma digoxin concentrations when taking normal doses of Boots digoxin and was able to tolerate the very high dosage of 2.0mg/day. It had been shown that some patients with intestinal malabsorption syndromes could have low plasma digoxin concentrations (Heizer et al., 1971). However a generalised malabsorption state had been excluded in M.P. Craig and Lown (1958) had reported a case of a woman whose atrial fibrillation was controlled by intravenous digoxin when high oral doses had failed - they suggested that impaired absorption of digoxin might have been present but did not investigate this further. A case had also been previously described in which digitalisation was affected by increased conversion of digoxin to the cardio-inactive metabolite dihydrodigoxigenin (Luchi and Gruber, 1968), a metabolite which would not have been detected by the radioimmunoassay. This patient had tolerated oral digoxin doses of 2 - 3mg/day but exhibited abnormal behavioural signs when on these high doses. An element of impaired bioavailability cannot, in retrospect, be excluded in Luchi and Gruber's patient and indeed, from the urinary digoxin excretion data, appears likely. Abnormal metabolism was not the explanation in the case of M.P. since her elimination rate of digoxin after an intravenous dose was normal. Her digoxin absorption curves alter the 0.5mg doses of Boots digoxin were very abnormal compared with results in normal subjects and indicated that the defect lay in her digoxin absorption. The increase in her steady state plasma digoxin concentrations after changing to Lanoxin, and later to capsules containing powders of her Boots tablets, indicated that her capacity to absorb digoxin was not defective. M.P. had been started on quinidine therapy in the interval between using the Boots and Lanoxin formulations. Although not considered of importance at

the time, the recent discovery that quinidine increases plasma digoxin concentrations by up to 100% (Mannimen and Nyberg, 1981) would now indicate that this drug interaction could account for some of the increase in digoxin concentration. It would not however cause the observed differences in absorption profile and did not apply to the increase after changing to the capsules.

Patient L.S. had shown a significant improvement in the ventricular rate of his atrial fibrillation after changing to Lanoxin. His plasma digoxin concentration increased by 68%. With the number of estimations performed, this increase just failed to reach statistical significance, although the plasma digoxin concentrations during use of the capsules were significantly higher than those found when he used the Harker-Stagg tablets in their original form.

Although in patient M.P. the Boots brand of digoxin tablet had been shown to have impaired bioavailability compared with Lanoxin, a similar result was not found in the other individual patient studies when the Boots brand had been used. In animal experiments digoxin was absorbed by passive diffusion through the wall of the small intestine (Caldwell et al., 1969, Greenberger et al., 1969). It seemed a possibility that some patients had a relatively small capacity for digoxin absorption by virtue of the length, nature or motility of their small intestine and this restricted capacity could make them more sensitive than others to any delay in release of digoxin from its formulation. This concept had some support from the slight trend in the individual patient studies whereby those with lower digoxin levels on their original tablets showed a greater increase after changing to Lanoxin. Since digoxin was thought to be

absorbed by simple passive diffusion it seemed most strange that M.P. could have such impaired digoxin absorption while able to absorb other substances quite normally. The explanation clearly might lie with the formulation of her tablets but standard pharmaceutical tests had not shown any formulation abnormality. Her tablets, and those of L.S., met the then current criteria of the British and American pharmacopoeiae. However when crushing the original tablets of M.P. and L.S. and administering the fine powder within a capsule produced more rapid absorption and higher steady state plasma digoxin concentrations, this demonstrated that there was a large effect on absorption by the method of formulation. We concluded at this time that the formulation effect might be due to failure of the tablets to disintegrate to fragments of sufficiently small size for rapid dissolution or to the influence of particle size (Shaw, Howard and Hamer, 1972).

The results of the survey of out-patients had shown that for patients taking 0.125 - 0.5mg/day of digoxin there was no difference in the steady state plasma digoxin concentrations obtained with Lanoxin and other brands. There had been a significant difference in the plasma digoxin concentrations of the out-patients who had been using Lanoxin and other brands at a dosage of 0.625 - 0.75mg/day. However the number of patients in the 'other brands' group was only 3, one of whom was L.S., who had attended the clinic for a routine cardiac follow-up. Hibble, Isaacs and Grahame-Smith (1972) published results of absorption curves recorded in four subjects with three brands of digoxin, one of which was Lanoxin. They found no difference in the areas under the five hour curves. This evidence suggested that

patients like M.P. and L.S. were uncommon. The few patients who were unusually poor absorbers of digoxin would tend to have been put on higher doses of digoxin, and this might explain the difference found in the 0.625 - 0.75mg/day group.

It was puzzling that the out-patients had plasma digoxin concentrations much lower than those reported by Chamberlain et al., (1970) and Evered, Chapman and Hayter (1970) and that so many had a concentration below the range of 1 - 2 ng/ml. At a daily dose of 0.5mg/day Chamberlain and colleagues found a mean plasma digoxin concentration of 1.5 S.D. 0.4ng/ml, while Evered's group found a mean concentration of 1.86 S.D. 1.23ng/ml. It was possible that our patients were poor in their compliance with their prescribed dosage. Heart failure patients have been found to be relatively good at complying with their digoxin regimes (Brook et al., 1971) and patients whom I questioned about compliance maintained that they had been very regular in taking their digoxin tablets.

The reason for the unexpectedly low plasma digoxin concentrations with Lanoxin became clear at the beginning of August 1972. Burroughs Wellcome Ltd announced that an alteration in the manufacturing process of Lanoxin had resulted in a major change in its bioavailability (Lancet, 1972). It was later explained that a combination of <u>in vitro</u> tablet analyses and results of absorption tests with Lanoxin batches in four normal subjects had led the company to realise that a change in Lanoxin tablet production in late 1969 had resulted in a decrease in bioavailability. It was estimated that this change caused the bioavailability of Lanoxin to be halved (Munro-Faure et al., 1974). There had in addition been a return in May 1972 to the original

Lanoxin production method which had pertained up to 1969 (Munro-Faure et al., 1974) i.e. during the period late 1969 to May 1972 Lanoxin tablets had been markedly reduced in bioavailability. By the time the changes in Lanoxin bioavailability were noted, supplies of the high bioavailability formulation had already been distributed to many retail pharmacists.

Burroughs Wellcome were faced with the dilemma of having two different Lanoxin formulations in distribution simultaneously. The company chose to recall the batches of lower bioavailability and wrote to all medical practitioners to explain their action (Figure 6). It is perhaps unfortunate that these announcements and newspaper reports (The Times, 1972, Daily Mirror, 1972) did not refer to the 1969 change and this resulted in some confusion about dosage. In view of the differences which now existed between Lanoxin and other brands (Isaacs et al., 1972, Shaw et al., 1972) the Committee on Safety of Medicines advised pharmacists to dispense Lanoxin only when it had been specified on the prescription or when the physician had indicated that dosage had been reassessed (Pharmaceutical Journal, 1972a).

Burroughs Wellcome had acted swiftly to alert physicians about the change in Lanoxin but the company was uncertain about the exact magnitude of the change. Differences between formulations had become evident but no pharmacopoeal standard was available to ensure consistent bioavailability. A digoxin bioavailability problem of awesome proportions had appeared.

Burroughs Wellcome & Co Medical Sales Division
The Wellcome Foundation Ltd
Temple Hill Dartford Kent DA1 5AH

Wellcome

telegrams Tabloid Dartford
telex 896758
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Dear Doctor,

WARNING – DIGOXIN

There is now evidence which suggests that the nominal content of digoxin in a tablet is an inadequate indicator of potency. The Committee on the Safety of Medicines is aware of this fact.

Tablets of different brands, all with a nominal content of 0·25 mg digoxin, may produce different therapeutic responses. When the substance is relatively insoluble, as is digoxin, the extent to which it is absorbed can be affected considerably by the method of formulation and method of manufacture of the tablet. Until very recently such differences were not detectable by assay procedures, but revised quality control procedures have enabled us to manufacture LANOXIN* tablets with a more predictable clinical response. It is now apparent, however, that these tablets, whilst still containing 0·25 mg digoxin, have approximately double the effective potency of earlier batches.

IN THESE CIRCUMSTANCES, IT IS IMPORTANT TO REVIEW THE DOSAGE OF ALL PATIENTS RECEIVING LANOXIN TABLETS. This is of particular importance with certain classes of patient. These classes include children, the elderly and others with impaired renal function, patients who are also receiving diuretics without potassium supplements, and patients receiving doses of 0·5 mg LANOXIN or more daily. IN THESE PATIENTS IT MAY BE ADVISABLE TO CONSIDER REDUCING THE DOSE OF LANOXIN BY HALF.

The bio-availability of digoxin from LANOXIN tablets may also be greater than that from other digoxin tablets, which may in turn differ from each other. Patients stabilised on one brand of digoxin could be improperly maintained if subsequently treated with another brand.

We have, in consultation with the Committee on the Safety of Medicines, decided to recall all stocks of LANOXIN tablets manufactured before May 1972 and to replace them with tablets of the new standard.

Yours faithfully,

BURROUGHS WELLCOME & CO.

Note LANOXIN can be identified by the word Wellcome on one side together with a code Number, X3A for the 0·25 mg product and U3A for the 0·0625 mg Paediatric/Geriatric product

DISPENSING DOCTORS should examine their stocks of both products and compare them with the Batch Numbers below. All stock not bearing one of these numbers should be returned to their wholesaler who will replace with material from the new batches.

LANOXIN P.G. Tablets	LANOXIN Tablets 0·25 mg	
1486-X	1482-X	1579-X
1531-X	1/1482-X	1538-X
1559-X	1483-X	1621-X
1571-X	1/1483-X	1620-X
1594-X	2/1483-X	1623-X
1740-X	1484-X	1666-X
1748-X	1485-X	1667-X
1749-X	1546-X	1745-X
1750-X	1580-X	1746-X
		1767-X

Subsequent batches of new material will have Batch Numbers greater than those listed.

*Trade Mark

GS 6602/86

Figure 6 Letter sent to medical practitioners by Burroughs Wellcome in August 1972.

CHAPTER 3

STUDIES ON THE EFFECT OF THE CHANGE IN THE MANUFACTURING TECHNIQUE OF LANOXIN

INTRODUCTION

After the announcement that the Lanoxin brand of digoxin tablet had undergone an unexpected increase in bioavailability in May 1972, I wanted to establish how the steady state plasma digoxin concentrations in patients using this newer formulation ('newer Lanoxin') compared with those obtained with the 'older Lanoxin' manufactured just before the change. It would also clearly be of interest to compare results from these formulations with Lanoxin produced prior to the initial alteration in manufacturing technique in 1969. Fortunately steady state plasma digoxin concentrations in cardiac out-patients at St. Bartholomew's Hospital had been recorded early in 1969 by Chamberlain et al., (1970). The patients in my survey of out-patients formed a group who had been using 'older Lanoxin'. Since an accurate estimation of the bioavailability of the newer Lanoxin was not known at this time, I felt that a randomised crossover study at fixed dosage was not justifiable because of the risk of inducing digitalis toxicity. The patients who received the newer Lanoxin were therefore collected in two ways:- (1) a group of out-patients living near the hospital would be changed from the older Lanoxin to the newer Lanoxin. Dosage would be reduced if previously they had been taking an unusually high dose of digoxin relative to their weight and renal function.
(2) Patients in the medical and surgical wards of St. Bartholomew's Hospital who were receiving digoxin would be put on the newer Lanoxin formulation, with dosage adjusted, when necessary, in a similar manner.

In order to observe the peak plasma digoxin concentrations after oral doses of older and newer Lanoxin, absorption curves were measured in myself and my colleague, Michael Howard.

I obtained samples of tablets from a range of companies for *in vitro* testing to investigate how newer Lanoxin compared with digoxin tablets of other manufacturers.

METHODS

Patients

a) Out-patients

One hundred and eleven patients had now been surveyed at the out-patient clinic. Sixty had used Lanoxin and 51 had used other brands. In 2 cases, one from each group, a digoxin dose had been taken immediately before the clinic visit and it was not possible to collect a blood sample at over 6 hours after their preceding dose and these patients were excluded. Three patients had taken their last dose 10 - 18 hours before blood sampling, with the interval being 6 - 10 hours in the remainder. The brand of tablet was identified as described in Chapter 2.

To compare the plasma digoxin concentrations of these patients with the results obtained by Chamberlain and colleagues in early 1969 it was necessary to restrict the analysis to patients with normal or near normal renal function as Chamberlain had studied only patients with good renal function. Thirty patients with a blood urea greater than 40mg/100ml were therefore excluded. Five patients taking 0.125mg/day of digoxin and 1 patient taking 1mg/day were also excluded as Chamberlain et al., had not included patients at these dosage levels. Of the 73 patients remaining 38 had used the older Lanoxin and 35 had used other brands.

Twenty of the out-patients with good renal function had plasma digoxin concentrations measured after changing to newer Lanoxin at the same dosage. Eight of these 20 patients had previously used a brand other than Lanoxin: they were given a supply of newer Lanoxin (batch 1579X) and had their plasma digoxin concentration measured one week later at 6 - 10 hours after their last dose. Twelve of the 20 patients had previously been using Lanoxin: they were given first a supply of older Lanoxin (batch 0953X) and had a blood sample taken for plasma digoxin concentration estimation one week later. They were then changed to newer Lanoxin (batch 1579X) at the same dosage and returned for measurement of digoxin concentration after one week.

b) In-patients

I carried out a review of all the patients in the medical and surgical wards of the hospital on 8th August 1971, and recorded details of those on digoxin treatment. Three different batches of Lanoxin were in use in the wards on that date - 0953X (older Lanoxin) and 1483X and 1579X (both newer Lanoxin). The bottle of digoxin in each ward had the batch number printed on its label and the date of supply of the bottle to the ward was available from the pharmacy records. It was therefore possible to establish which formulation had been used by each in-patient during the preceding week. With the consultants' permission, each patient was changed to an appropriate dose of newer Lanoxin. Twelve in-patients on digoxin had a blood urea of less than 40mg/100ml. Five had already been receiving the newer Lanoxin for at least one week - a blood sample for plasma digoxin estimation was taken 6 - 12 hours after their preceding dose. Seven had been receiving older Lanoxin for a week or more. Measurement of their state steady plasma digoxin concentration was made prior to

changing their therapy to newer Lanoxin (batch 1579X) at the same dosage. Plasma digoxin concentration was measured again after one week on the newer formulation.

All of the out-patients and in-patients had a 30 second lead II rhythm strip recorded at the time of each blood sampling.

Lanoxin tablets

The results of pharmaceutical tests on tablets of batch 1579X newer Lanoxin were provided for me by H. Greer of the Pharmaceutical Development Department, The Wellcome Foundation Ltd, Dartford, Kent:-

Mean tablet weight : 112mg S.D. 1.1mg (sample of 20 tablets)

Content : bulk assay : 101.2% of stated dose

individual tablet assay : 100.8% S.D. 1.9%
(sample of 20 tablets)

Tablet hardness (Monsanto Tester) : 3 - 4Kg

Disintegration time : 2 minutes

The dissolution test results of this batch, and of batch 0953X (older) Lanoxin, is included in the results section.

Disintegration and dissolution rate tests

Batches of digoxin tablets were obtained from 15 different manufacturers. This list of manufacturers had been compiled during brand identification in the out-patient survey. The disintegration and dissolution rate of these tablets, and of batches 1579X (newer) Lanoxin and 0953X (older) Lanoxin were measured by Mr. A.C. Caws of the Central Analytical Laboratories (Chemical) of The Wellcome Foundation Ltd. The disintegration rate method was that of the British Pharmacopoeia (1968). The dissolution rate method was based on that of the United States Pharmacopeia and has been described in detail by Caws, Jenkins and McCrerie (1974). Six tablets of 0.25mg strength

were placed in a stainless steel basket set to rotate at 120rpm in 500ml of 0.6% hydrochloric acid. Samples of the fluid were removed at intervals for measurement of digoxin concentration by the fluorimetric method.

Absorption curves

Plasma digoxin concentrations were measured from 0 - 8 hours after 0.5mg oral doses of digoxin in two normal subjects - MRH (68Kg) and TRDS (65Kg). The subjects had fasted overnight prior to taking the dose along with 50ml of water. Two tablets formations were used - older Lanoxin of batch 0953X and newer Lanoxin of batch 1579X. A liquid formulation of Lanoxin was also taken orally as a 0.5mg dose of Lanoxin solution (lot 80601) marketed for parenteral injection. The doses were given in randomised order and at least 3 weeks elapsed between doses. The subjects were ambulant after the doses were taken.

RESULTS

The steady state plasma digoxin concentrations recorded in the 38 out-patients using older Lanoxin and the 35 out-patients using other brands are given in Table 4. This Table also gives the digoxin concentration results of the 32 in-patients and out-patients who had received newer Lanoxin. The Table includes the digoxin concentrations recorded by Chamberlain et al., (1970) in similar patients in 1969, prior to the initial change in Lanoxin manufacturing technique. Older Lanoxin and the other brands produced similar plasma digoxin concentrations ($p > 0.05$ for all dosage groups, Student's paired t test). Newer Lanoxin gave significantly higher plasma digoxin concentrations than older Lanoxin : 86% higher at a dosage of 0.25 - 0.37mg/day ($p < 0.01$), 69% higher at a dosage of 0.5mg/day ($p < 0.01$) and 74% higher for a dosage of 0.625 - 0.75mg/day

TABLE 4

PLASMA DIGOXIN CONCENTRATIONS (MEAN ± S.D.) RECORDED IN OUT-PATIENTS WITH NORMAL OR NEAR NORMAL RENAL FUNCTION (BLOOD UREA LESS THAN 40mg/100ml) DURING USE OF OLDER LANOXIN, NEWER LANOXIN AND OTHER BRANDS. TABLE INCLUDES FOR COMPARISON THE PLASMA DIGOXIN CONCENTRATIONS RECORDED IN SIMILAR PATIENTS USING LANOXIN IN EARLY 1969

Brand	Daily dose of digoxin (mg/day)		
	0.25 - 0.375	0.5	0.625 - 0.75
Other brands	0.60 ± 0.25 n = 15	0.93 ± 0.31 n = 14	1.02 ± 0.69 n = 6
Older Lanoxin	0.56 ± 0.23 n = 12	0.94 ± 0.33 n = 17	1.18 ± 0.39 n = 9
Newer Lanoxin	1.04 ± 0.26 n = 15	1.59 ± 0.56 n = 15	2.05 ± 0.07 n = 2
1969 Lanoxin (Chamberlain et al., 1970)	0.9 ± 0.4 n = 21	1.5 ± 0.4 n = 31	2.1 ± 0.6 n = 11

($p < 0.05$). Digoxin concentrations with newer Lanoxin were very similar to those found with Lanoxin in 1969 by Chamberlain et al. The differences between these formulations are illustrated in Figure 7.

The results found in the 19 patients who received both older and newer Lanoxin are given in Table 5. The mean plasma digoxin concentration when using older Lanoxin was 0.86ng/ml (S.D. 0.28) which increased by 70% to 1.46ng/ml (S.D. 0.54) after the change to newer Lanoxin. This difference in plasma digoxin concentration was statistically significant ($p < 0.01$, paired t test). The increase recorded in individual subjects varied considerably. In the 0.5mg/day group the increase varied from 0% to 317%.

None of these patients developed digitalis toxicity as a result of changing from older to newer Lanoxin. Plasma digoxin concentrations found in the out-patients survey had tended to be below the range of 1 - 2ng/ml (Figure 8) and 75 of the 109 patients (69%) had a digoxin concentration of less than 1ng/ml. There was also a high incidence of poorly controlled atrial fibrillation. Table 6 shows the ventricular rates recorded at rest in the 74 patients who had atrial fibrillation. In 51% the ventricular rate was greater than 90 beats per minute. Fourteen patients (19%) had been prescribed a beta-adrenoreceptor blocking drug to help in the control of heart rate. The incidence of digitalis toxicity was also very low. Of the 109 patients, 14 had, at their first out-patient interview, an arrhythmia classified as possibly due to digitalis toxicity according to the criteria of Beller et al., (1971). To determine if the arrhythmia was due to toxicity or to heart disease each of these 14 patients had further observations after their digoxin dosage had been either reduced or increased. The decision to reduce or increase digoxin dose was based

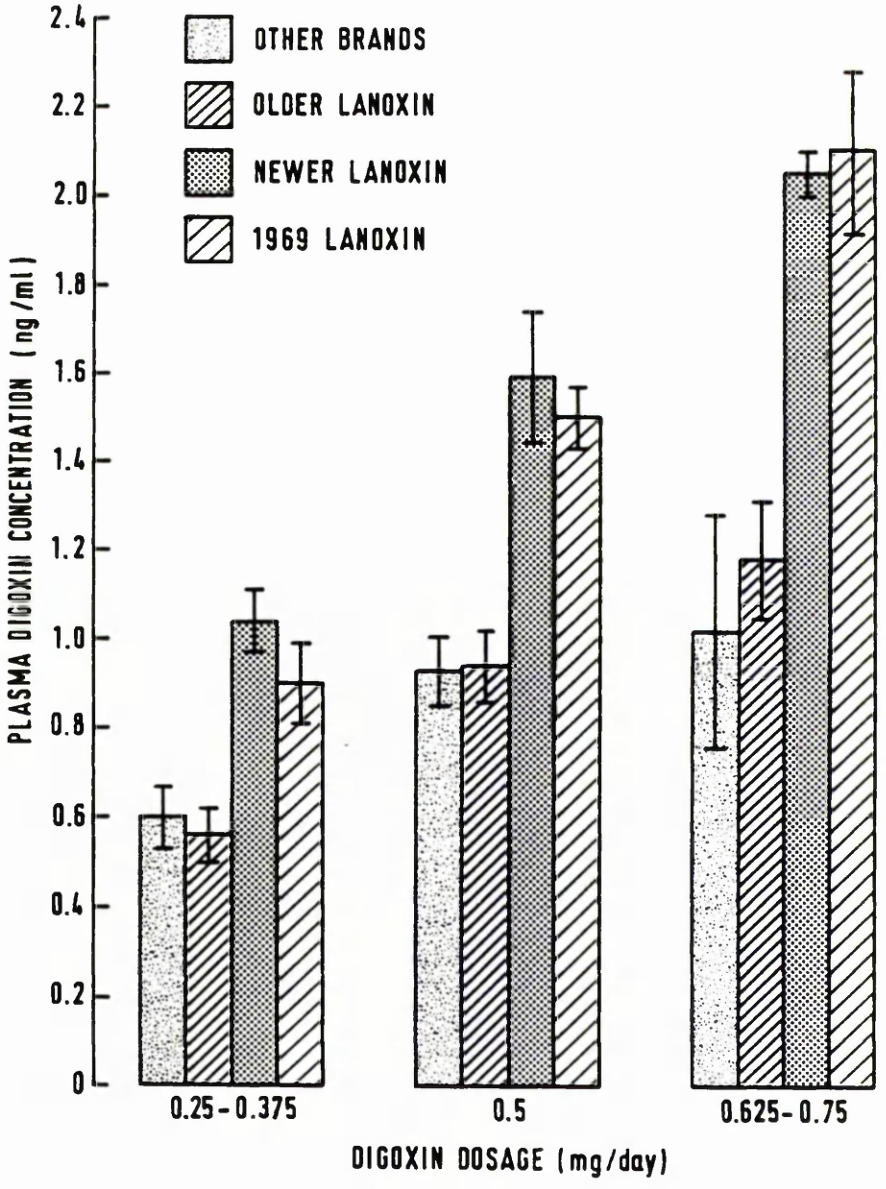

Figure 7 Steady state plasma digoxin concentrations (mean ± SEM) recorded during use of older Lanoxin, newer Lanoxin, other brands and Lanoxin produced prior to its change in 1969. All patients had blood urea of less than 40mg/100ml.

TABLE 5

PLASMA DIGOXIN CONCENTRATIONS IN PATIENTS WHO HAD USED BOTH OLDER AND NEWER LANOXIN: ALL PATIENTS HAD NEAR NORMAL RENAL FUNCTION

Case No.	Body weight (Kg)	Daily dose of digoxin (mg)	Plasma digoxin concentration when using older Lanoxin (ng/ml)	Plasma digoxin concentration when using newer Lanoxin (ng/ml)
1	65	0.25	0.6	1.1
2	55	0.375	0.9	1.4
3	52	0.375	0.7	1.5
4	50	0.25	1.0	1.2
5	43	0.25	1.0	1.0
6	67	0.25	1.0	0.8
7	70	0.5	0.25	0.8
8	93	0.5	0.5	1.2
9	60	0.5	0.6	2.5
10	63	0.5	0.6	0.9
11	58	0.5	0.7	1.7
12	68	0.5	0.8	1.5
13	71	0.5	0.8	1.3
14	60	0.5	1.0	1.4
15	55	0.5	1.0	1.0
16	32	0.5	1.3	2.7
17	80	0.5	1.3	1.7
18	70	0.75	0.9	2.1
19	85	0.75	1.3	2.0
Mean ± S.D.			0.86 ± 0.28	1.46 ± 0.54

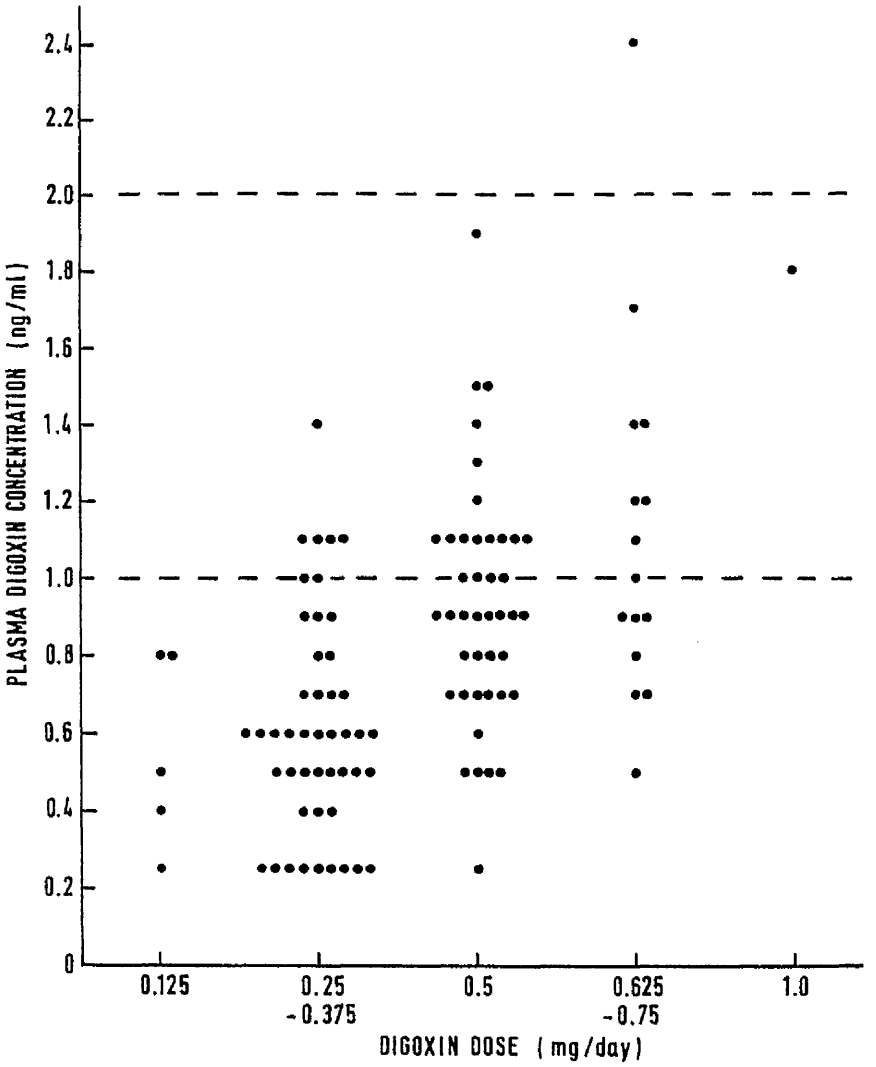

Figure 8 Plasma digoxin concentrations recorded in 109 out-patients prior to the introduction of newer Lanoxin.

TABLE 6

THE RESTING VENTRICULAR RATES IN 74 OUT-PATIENTS WITH ATRIAL FIBRILLATION PRIOR TO THE INTRODUCTION OF NEWER LANOXIN

RESTING VENTRICULAR RATE (BEATS/MIN)	LESS THAN 70	70 - 79	80 - 89	90 - 99	GREATER THAN 100
NUMBER OF PATIENTS	14	10	12	18	20
PERCENTAGE OF TOTAL	19	14	16	24	27
NUMBER OF PATIENTS USING A BETA ADRENORECEPTOR BLOCKING DRUG IN ADDITION TO DIGITALIS	0	2	5	3	4

on the plasma digoxin concentration and the dosage used. The arrhythmias were:- multiform ventricular ectopic beats at more than 5 per minute - 7 patients; unifocal ventricular ectopic beats of more than 5 per minute - 4 patients; supraventricular ectopic beats at more than 5 per minute - 2 patients; nodal rhythm - 1 patient. The patient with a chronic nodal focus had this arrhythmia persist even after digoxin was stopped. Five of the patients with ectopic beats had their ectopy cease or reduce in frequency when digoxin dosage was increased. In 7 patients the ectopy persisted despite reduction or cessation of digoxin dosage. It was concluded therefore that in 12 of these 13 patients the arrhythmias were due to their heart disease rather than to digitalis toxicity. Only one patient had probable toxicity : in her case multifocal ventricular ectopic beats progressively increased in frequency as digoxin dosage and plasma digoxin concentrations increased, culminating in coupled ectopic beats at a plasma digoxin level of 2.5ng/ml.

Tablet dissolution rates

The results of the dissolution rate tests are given in Table 7. There was a wide variation between brands. The percentage of the stated dose in solution by 60 minutes varied from 33 to 118%.

Absorption curves with older and newer Lanoxin

The plasma digoxin concentrations recorded in the 2 normal subjects after 0.5mg oral doses of older Lanoxin, newer Lanoxin and Lanoxin solution are shown in Figure 9. Peak plasma concentrations after the solution (2.1 and 3.4ng/ml) were similar to those after newer Lanoxin (2.3 and 2.7ng/ml) and considerably higher than the peak concentrations after older Lanoxin (0.9 and 0.9ng/ml).

TABLE 7

DISINTEGRATION RATES AND DISSOLUTION RATES
OF OLDER AND NEWER LANOXIN AND 15 OTHER BRANDS
MARKETED IN THE UNITED KINGDOM IN EARLY 1972

Manufacturer	Disintegration time (minutes)	% of stated digoxin dose in solution	
		at 15 minutes	at 60 minutes
Boots	6	21	37
Evans Medical	17	22	42
Kerfoot	3	27	43
Martindale Samoore	4	34	56
Ward Blenkinsop	3	16	33
Cartwright	4	41	72
Oppenheimer	2	45	65
Cox	1	90	118
Kirby	7	78	99
Halewood	5	30	52
Clonmell	< 15	-	59
Interalia	< 15	-	51
Nativelle	< 15	-	87
McCarthy	< 15	-	70
Matthews & Wilson	< 15	-	52
Older Lanoxin	< 15	27	48
Newer Lanoxin	2	87	97

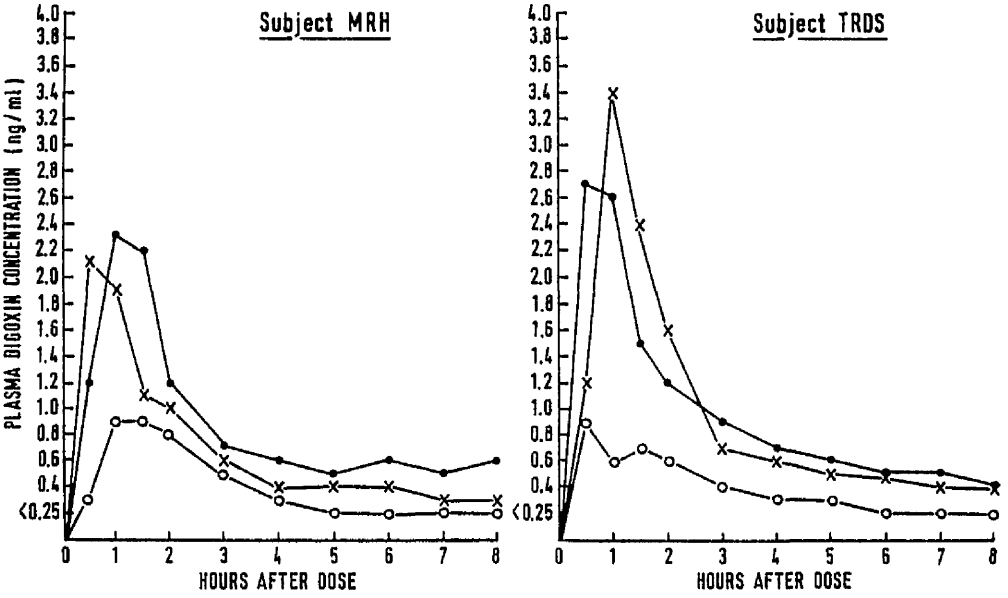

Figure 9 Plasma digoxin concentrations recorded in normal subjects M.H.R. and T.R.D.S. after 0.5mg oral doses of older Lanoxin (open circles), newer Lanoxin (closed circles) and Lanoxin solution (crosses).

DISCUSSION

The object of this part of my studies had been to assess how the bioavailability of newer Lanoxin compared with (1)'older Lanoxin' and (2) Lanoxin produced before the first manufacturing change of 1969. It was necessary to do this in a way which would protect patients from developing digitalis toxicity. It was also important to be able to obtain results quickly as we were faced at that time with the clinical problem of modifying the digoxin therapy of large numbers of cardiac patients as a consequence of the latest Lanoxin alteration. These objectives seemed to be met best by measuring the steady state plasma digoxin concentrations in a further group of patients rather than by undertaking a series of single dose absorption-curve studies in normal people, although such studies would clearly also be required before the bioavailability of Lanoxin was fully characterised. I chose the method which I felt suited best the objectives in view and the facilities available: the hospital provided contact with many patients on digoxin treatment but had very limited access to normal subjects able to take part in prolonged bioavailability studies. This was a policy which was to be followed in my subsequent bioavailability experiments.

The patients who received both older Lanoxin and newer Lanoxin at the same nominal dose had shown a mean increase of 70% in plasma digoxin concentration after changing to the newer formulation. Differences of similar magnitude were found at each dosage level in the patient survey groups. These results confirmed that newer Lanoxin gave considerably greater absorption of digoxin, but the increase was slightly less than the two-fold difference initially estimated by Burroughs Wellcome. Falch, Teien and Bjerkelund (1973) reported results

obtained with older and newer Lanoxin in patients and normal volunteers. The newer Lanoxin gave much higher peak plasma digoxin concentrations during the absorptive phase and urinary excretion of digoxin was 40% greater with the newer formulation. However they found only a 10% increase in steady state concentrations in a group of 14 patients. In this group the older Lanoxin had given a relatively high mean plasma digoxin concentration of 0.94ng/ml for a dose of 0.25mg/day. Patients at a higher dosage were not included and it was not clear if the authors had selected for the trial only patients on a low dosage, who may have had a good absorptive capacity for slowly released digoxin. Whiting, Rodger and Sumner (1972) compared a group of 30 patients on 0.5mg/day of older Lanoxin with a group of 24 patients taking 0.25mg/day of newer Lanoxin. Taking into account the change in dosage, steady state plasma digoxin concentrations indicated that newer Lanoxin had 58% greater bioavailability. An average increase of Lanoxin bioavailability of 178% was found by Stewart and Simpson (1972), using steady state measurements in an undefined population. Manninen, Ojala, and Reissell (1972) noted a 28% increase in steady state digoxin concentration in a small group of 8 patients. In a single dose study in normal volunteers, Johnson et al., (1973) found that the area under the 50 hour absorption curve was 84% greater with newer Lanoxin compared with older Lanoxin, and 4 day urinary excretion was 100% higher. The difference in magnitude of change between studies must reflect a number of factors - design of the study, subject population and the particular batches of older Lanoxin used (the dissolution rate of batches of older Lanoxin varied from 43% - 64%, according to Munro-Faure et al., (1974). The mean increase found in these reports was 73%. For individual patients the

increase in Lanoxin bioavailability could be as great as several-fold. In this circumstance there would be a considerable risk of toxicity if the patient changed from a low to a high bioavailability digoxin formulation at a dosage chosen to give adequate therapeutic affect during use of the low bioavailability product. Fortunately for most patients, physicians seemed to have maintained a conservative approach to digoxin dosage during 1969 - 1972 and underdigitalisation with low-bioavailability tablets was the commoner phenomenon. This was illustrated by the poor control of atrial fibrillation and the lack of toxicity seen in the out-patient survey.

Reports of differences in bioavailability of digoxin tablet brands were subsequently reported from many countries: e.g. Finland (Manninen et al., 1971, Karjalainen, Ojala and Reissell, 1974 Lisalo & Ruikka, 1974), Sweden (Redfors et al., 1973, Nyberg et al., 1977), Denmark (Steiness, Christensen and Johansen, 1973), Switzerland (Beveridge et al., 1975) and Australia (McCredie et al., 1973).

The newer Lanoxin produced in Britain was subsequently shown to have the same bioavailability as a liquid formulation. Johnson and Lader, (1974) found that the area under the 80 hour absorption curve and the 10 day urinary digoxin excretion were similar for single doses of newer Lanoxin and digoxin dissolved in an alcoholic solution. Manninen, Reissell and Ojala (1976) also found equivalent steady state plasma digoxin concentrations and urinary digoxin excretion at steady state during use of these formulations.

Lanoxin for the American market is produced within the United States. The American formulation did not undergo any changes in production technique during 1969 - 1972. However U.S. Lanoxin had a separate production technique and did not correspond exactly to either

older or newer British Lanoxin. Bioavailability studies in the United States subsequently established two points (1) differences in bioavailability existed between U.S. Lanoxin and other brands marketed in that country. (2) Absorption from U.S. Lanoxin was significantly less than from a digoxin solution.

In their initial report, Lindenbaum et al., (1971) had shown marked differences in the area under the 5 hour absorption curve for 4 U.S. digoxin brands. This indicated differences in the absorption rate but not necessarily in the extent of absorption (Sorby and Tozer, 1973). Lindenbaum's group did however later show differences in 24 hour urinary excretion of digoxin for these formulations and 24 hour excretion data has been found to correlate well with 6 day excretion results (Greenblatt et al., 1974). Wagner et al., (1973) showed that a U.S. digoxin tablet formulation which met all the U.S. Pharmacopeia standards had almost half the bioavailability of U.S. Lanoxin, as assessed by the area under the 96 hour absorption curves : this difference persisted even when the data was reinterpreted after extrapolation of the area-under-the-curve plots to infinity (Wagner and Ayres, 1977). Two-fold variation in steady state digoxin levels were recorded by Lindenbaum et al., (1973) in a study of 5 U.S. digoxin brands. Preibisz, Butler and Lindenbaum (1974) used both single dose and steady state plasma and urine concentration methods to compare 3 U.S. brands. The single dose studies gave greater differences than the steady state measurements, but even in the latter variation of nearly two-fold was found. They observed marked differences in bioavailability between different batches from the same manufacturer, as was also found by Lindenbaum (1975). In addition to differences in steady state, absorption curve and cumulative

urinary digoxin excretion with 2 U.S. brands, Fleckenstein, Kroening and Weintrant (1974) recorded parallel changes in systolic time intervals, reflecting different degrees of pharmacologic effect.

The submaximal bioavailability of U.S. Lanoxin has been documented by a number of studies. Huffman and Azarnoff (1972) used 10 day cumulative urinary excretion data and found U.S. Lanoxin gave 75% absorption compared with a solution. Vieweg and Sode (1973) suggested that U.S. Lanoxin and a solution had equivalent bioavailability but their data is insufficient for this conclusion. U.S. Lanoxin achieved 84% of the bioavailability of a digoxin exilir in the single dose studies by Greenblatt et al., (1973). In a steady state experiment which included chronic intravenous dosing Huffman, Manion and Azarnoff (1974) found the absolute bioavailability of digoxin solution to be 77% while that of U.S. Lanoxin was 62%. Lloyd et al., (1978) also showed that U.S. Lanoxin tablets were incompletely absorbed, using both single dose and steady state assessments.

In the United Kingdom the results in our out-patient survey confirmed that newer Lanoxin had much higher bioavailability than many of the other commonly used brands (Shaw, Howard and Hamer, 1974). The dissolution rate results on the 15 other brands also indicated that these brands differed from newer Lanoxin. A similar spectrum of dissolution rates in British digoxin tablets was found by Beckett and Cowan (1973) and Fraser, Leach and Poston (1974).

These studies had clearly demonstrated that a serious problem existed with digoxin tablet bioavailability. It remained to find a way to ensure equivalent and consistent bioavailability for digoxin tablets from all manufacturers.

CHAPTER 4

THERAPEUTIC NON-EQUIVALENCE OF DIGOXIN TABLETS IN THE UNITED KINGDOM AND ITS CORRELATION WITH TABLET DISSOLUTION RATE

INTRODUCTION

The experience gained from the study of older and newer Lanoxin and the data on the dissolution rates of other brands made it feasible to carry out a trial to compare different brands of digoxin tablets at fixed dosage. One problem in this type of study is the possibility of causing an unacceptable degree of toxicity or loss of therapeutic effect. However I had contact now with a large number of patients whose severity of heart disease, arrhythmia status, renal function and steady digoxin plasma digoxin concentrations were known. It therefore appeared ethical to enter these patients into a bioavailability trial at a dosage which one could expect with confidence would not cause serious toxicity nor deterioration of their clinical state. Now that the dissolution rates were known for a large number of digoxin tablet brands, it was possible to choose a selection of brands which would represent a full range of dissolution rates.

The design of a trial to compare the bioavailability of digoxin brands had to overcome one further problem. It would be desirable to compare the largest number of brands possible, but as most of the patients using digoxin were working or had commitments to their home, it was necessary to limit their hospital attendances for the trial to a degree which was acceptable to the patient. It was decided therefore to divide the patient population into two groups, each of which would receive four brands of digoxin tablet. The newer Lanoxin would be used by both groups, so that the data of the two groups could be combined.

The use of steady state plasma digoxin concentrations was chosen as the index of bioavailability as it appeared the most appropriate for the study of a large number of cardiac patients.

METHODS

Patients

Two groups, each of 20 subjects, were chosen from the 111 patients in the out-patient survey. These 40 patients were those who had attended the clinic most recently and who lived sufficiently close to the hospital to be able to undertake additional visits. A few patients who had had multiple attendances for previous digoxin bioavailability studies were not included. Each patient had agreed to take part after a full explanation of the trial and its procedures. The characteristics of the patients are given in Tables 8 and 9. Other medication remained unchanged throughout the trial. Two patients did not complete the trial period. Patient 1 of group 1 developed cholecystitis and was admitted to hospital. Patient 20 of group 2 was hospitalised because of myocardial infarction.

In group 1 there were 14 females and 5 males; group 2 had 15 females and 4 males.

Digoxin tablets

Seven brands of digoxin tablets were used in the trial.

They were:-	Group 1	Newer Lanoxin	(batch 1579X)
		Nativelle	(batch 369)
		Boots	(batch 12N)
		Older Lanoxin	(batch 0953X)
	Group 2	Newer Lanoxin	(batch 1579X)
		Macarthys	(batch S2435)

TABLE 8

CHARACTERISTICS OF PATIENTS IN GROUP 1

GROUP 1

Patient	Age (years)	Weight (Kg)	Height (cms)	Blood urea (mg %)	Serum creatinine (mg %)	Serum K (mE/l)	Diagnosis
1	72	59	152	35	1.1	3.6	VHD
2	62	68	178	49	1.3	3.4	HT
3	56	63	158	36	0.8	3.5	VHD
4	63	46	160	45	1.4	3.2	VHD
5	65	78	173	37	1.0	3.8	CM
6	61	71	169	22	1.0	3.3	IHD
7	63	65	107	22	1.0	3.4	VHD + HT
8	61	66	168	61	1.2	4.0	IHD
9	69	48	178	39	1.1	3.8	IHD
10	54	65	178	25	0.9	4.0	VHD
11	48	61	152	59	1.3	3.6	VHD
12	51	68	173	30	1.1	3.7	VHD
13	52	51	158	24	1.0	3.8	VHD
14	56	64	180	33	1.2	4.2	VHD
15	56	76	160	30	0.8	3.4	VHD
16	64	57	158	24	0.9	4.1	VHD + HT
17	47	54	99	30	0.8	3.6	VHD
18	66	76	165	32	0.9	3.6	IHD
19	66	46	160	49	1.0	4.0	HD
20	68	54	158	52	1.4	3.4	VHD + HT
MEAN	59.4	61.9	159.6	36.8	1.06	3.67	-
S.D.	6.7	10.1	21.7	12.4	0.20	0.29	-

(VHD = valvular heart disease, HT = hypertension, IHD = ischaemic heart disease, CM = cardiomyopathy)

TABLE 9

CHARACTERISTICS OF PATIENTS IN GROUP 2

GROUP 2

Patient	Age (years)	Weight (Kg)	Height (cms)	Blood urea (mg %)	Serum creatinine (mg %)	Serum K (mE/l)	Diagnosis
1	54	81	173	42	1.3	3.9	CP
2	71	56	165	29	1.0	3.2	VHD + IHD
3	65	71	182	39	1.6	4.1	VHD
4	55	67	170	30	0.9	4.1	VHD
5	51	64	168	25	0.8	3.8	VHD
6	52	64	160	33	1.0	3.7	VHD
7	72	51	149	40	1.2	3.8	CHD
8	63	48	156	33	1.1	4.3	IA
9	62	64	163	30	1.0	3.9	VHD
10	40	76	170	30	0.8	4.6	VHD
11	57	94	183	34	0.9	4.0	IHD
12	50	70	170	21	0.8	3.4	VHD
13	57	55	158	29	1.0	3.1	VHD
14	56	60	161	24	0.7	3.7	IA
15	48	54	158	37	0.8	4.2	VHD
16	67	54	160	35	0.9	3.8	VHD
17	58	64	158	27	0.9	3.5	VHD
18	57	57	170	51	1.3	4.8	VHD
19	51	57	156	48	1.2	3.7	VHD
20	64	76	173	34	1.1	3.9	IHD
MEAN	57.2	63.5	164.7	33.5	1.01	3.87	-
S.D.	8.1	11.3	8.9	7.9	0.23	0.43	-

(VHD = valvular heart disease, IHD = ischaemic heart disease, CHD = congenital heart disease, IA = idiopathic arrhythmia
CP = cor pulmonale)

Oppenheimer (batch 30005)

Cox (batch 212051)

The manufacturers had supplied, in confidence, the excipient substances of their tablets and an outline of their manufacturing process. All brands met the British Pharmacopoeia (1968) requirements for digoxin content.

Disintegration rate tests

These were carried out by K. Raymond at the Department of Pharmaceutics, School of Pharmacy, University of London, WC1 using the method of the British Pharmacopoeia (1968).

Dissolution rate tests

These were also carried out by K. Raymond. The method of the United States Pharmacopoeia (1970) was employed, using 600ml of distilled water (Raymond, Shotton and Patel, 1974). The basket was rotated at 120 revolutions per minute. Samples for digoxin assay were removed at 15, 30, 45, 60, 90 and 120 minutes. After filtering of the fluid sample through a Millipore filter (0.45μm pore size) digoxin was measured by the fluorimetric method of Jensen (1953). Six tablets were used for each measurement of dissolution rate and for each brand the dissolution rate test was performed on 3 separate occasions. The brands were coded and their exact identity was not known by K. Raymond at the time of performing the dissolution rate tests, although tablet markings prevented complete masking of brand identity.

Procedures

The patients were given coded bottles containing 50 tablets. The brands were used in randomised order. The code was retained by the pharmacy department and was released only after the dissolution rate and plasma digoxin concentration estimations had been completed. Each

patient took their digoxin dose in the evening to allow sufficient time between the last dose and the blood sample, which was taken on the following morning. Patients remained on each brand for a 2 week period at the end of which they attended for clinical examination, a venous blood sample for plasma digoxin concentration and recording of a 30 second electrocardiogram rhythm strip. A count of tablets remaining in the bottle was used to assess compliance with the dosage. Blood samples were always taken at least 10 hours after the preceding dose. The average interval from the dose was 13 hours (range 10 - 17) and varied little for individual patients.

RESULTS

The digoxin dosages and plasma digoxin concentrations recorded in the group 1 patients are in Table 10 and those of the group 2 patients are in Table 11. The mean digoxin dose used by the group 1 patients was 0.34mg/day (S.D. 0.12) and the mean dose of group 2 patients was 0.36mg/day (S.D. 0.20). The mean plasma digoxin concentrations found with each brand are given in Table 12. Analysis of variance showed that the differences within each group were statistically significant ($p < 0.01$).

The tablet counts showed that compliance with the dosage instructions had been good. The discrepancy between the number of tablets found to be remaining and the number expected to remain after correct dosing was less than 3% of the total amount of tablets prescribed.

The disintegration and dissolution rate test results are listed in Table 13. The dissolution rate profiles are illustrated in Figure 10. The reproducability of the dissolution rate measurements is shown in Table 14. Correlation coefficients were calculated for the percentage of stated dose in solution at each time interval and the mean plasma

TABLE 10

GROUP 1 PATIENTS : DAILY DIGOXIN DOSE AND PLASMA DIGOXIN CONCENTRATIONS RECORDED DURING USE OF 4 DIGOXIN TABLET FORMULATIONS

Patient	Dose (mg/day)	Plasma digoxin concentrations (ng/ml)			
		Newer Lanoxin	Older Lanoxin	Boots	Nativelle
1	-	-	-	-	-
2	0.25	1.0	0.5	0.8	1.0
3	0.5	1.3	1.1	1.0	1.5
4	0.25	1.9	1.4	1.6	2.3
5	0.5	1.2	0.6	0.9	1.3
6	0.25	0.6	0.3	0.4	0.4
7	0.5	1.9	1.0	1.2	1.3
8	0.25	1.2	0.8	1.0	1.1
9	0.25	0.7	0.6	0.7	0.8
10	0.5	1.2	0.8	0.9	0.9
11	0.25	1.2	1.0	0.9	1.4
12	0.25	1.0	0.8	0.9	0.8
13	0.5	2.6	2.2	1.9	2.1
14	0.5	1.5	1.1	1.3	1.5
15	0.25	0.8	0.5	0.7	0.7
16	0.25	1.3	0.4	0.6	0.9
17	0.5	1.8	1.4	1.8	1.5
18	0.25	0.8	0.6	0.6	0.9
19	0.25	0.9	0.9	0.9	0.9
20	0.25	1.8	1.2	1.2	1.5

TABLE 11

GROUP 2 PATIENTS : DAILY DIGOXIN DOSE AND PLASMA DIGOXIN CONCENTRATIONS RECORDED DURING USE OF 4 DIGOXIN TABLET FORMULATIONS

Patient	Dose (mg/day)	Plasma digoxin concentrations (ng/ml)			
		Newer Lanoxin	Cox	Oppenheimer	Macarthys
1	0.25	0.7	0.7	0.7	0.4
2	0.25	0.6	0.4	0.4	0.7
3	0.25	1.0	0.8	1.1	0.7
4	0.5	1.8	0.5	0.8	1.5
5	0.5	1.5	1.4	1.1	1.3
6	0.5	1.2	1.2	1.4	1.4
7	0.25	1.0	0.7	0.6	0.6
8	0.25	1.1	0.6	1.0	1.3
9	0.25	0.7	0.8	0.7	1.1
10	0.25	0.6	0.5	0.4	0.6
11	1.0	1.1	0.9	1.0	1.0
12	0.5	1.3	1.1	1.2	1.3
13	0.5	0.9	0.8	0.8	1.0
14	0.5	1.2	0.9	1.1	1.3
15	0.25	1.1	0.8	1.0	0.7
16	0.25	1.0	0.6	0.6	0.7
17	0.25	1.1	0.6	0.7	0.8
18	0.25	0.7	0.4	0.5	0.6
19	0.125	0.6	0.5	0.6	0.5
20	-	-	-	-	-

TABLE 12

MEAN PLASMA DIGOXIN CONCENTRATIONS (± S.D.) FOUND WITH THE SEVEN BRANDS OF DIGOXIN TABLET. THE FIGURE IN PARENTHESIS GIVES THE MEAN CONCENTRATION EXPRESSED AS A PERCENTAGE OF THE MEAN CONCENTRATION FOUND WITH NEWER LANOXIN IN THE PATIENTS OF THAT GROUP

	Plasma digoxin concentrations (ng/ml) : means ± S.D.			
Brand	Newer Lanoxin	Nativelle	Boots	Older Lanoxin
Group 1	1.30 ± 0.51 (100)	1.20 ± 0.47 (92)	1.02 ± 0.40 (78)	0.91 ± 0.45 (70)
Brand	Newer Lanoxin	Macarthys	Oppenheimer	Cox
Group 2	1.01 ± 0.32 (100)	0.92 ± 0.35 (91)	0.82 ± 0.28 (81)	0.75 ± 0.27 (74)

TABLE 13

DISSOLUTION RATES AND DISINTEGRATION TIMES OF THE 7 FORMULATIONS OF DIGOXIN TABLET.
THE CORRELATION COEFFICIENTS ARE FROM THE LINEAR REGRESSION ANALYSIS OF THE PERCENTAGE OF DIGOXIN CONTENT IN SOLUTION AND THE PLASMA DIGOXIN CONCENTRATIONS EXPRESSED AS A PERCENTAGE OF THE MEAN LEVEL FOUND WITH NEWER LANOXIN

Brand	Disintegration time (seconds)	Dissolution rate: Percentage of stated dose in solution at: 15 min	30 min	45 min	60 min	90 min	120 min
Newer Lanoxin	111	62	86	94	100	104	106
Nativelle	40	42	57	62	67	70	73
Macarthys	210	36	48	53	57	63	65
Oppenheimer	136	39	44	51	52	55	60
Boots	263	22	32	42	41	47	52
Cox	108	24	32	37	38	41	44
Older Lanoxin	161	27	37	45	48	54	54
Correlation coefficient: % in solution - plasma digoxin concentrations	-	0.87	0.89	0.86	0.87	0.86	0.88

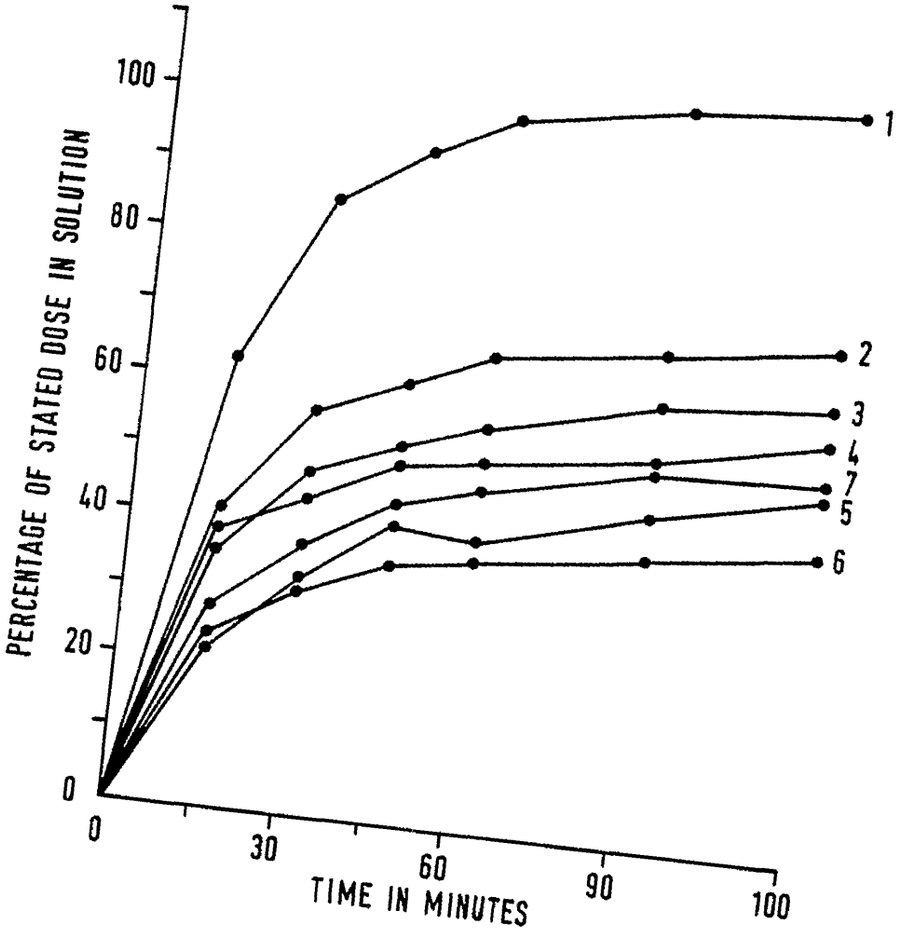

Figure 10 Dissolution rates of the seven types of digoxin tablet. 1 = Newer Lanoxin, 2 = Nativelle, 3 = Macarthy's, 4 = Oppenheimer, 5 = Boots, 6 = Cox, 7 = Older Lanoxin.

TABLE 14

RESULTS OF INDIVIDUAL DISSOLUTION RATE TESTS OF THE 7 FORMULATIONS OF DIGOXIN TABLET

Brand	Percentage of stated dose in solution at:-																	
	15 min			30 min			45 min			60 min			90 min			120 min		
Newer Lanoxin	88	17	81	92	78	89	103	89	90	110	95	96	107	110	96	100	95	96
Nativelle	49	26	50	58	52	62	64	60	63	69	64	67	72	66	73	76	71	73
Macarthys	42	25	42	51	46	48	56	48	56	58	54	60	66	58	64	67	60	68
Oppenheimer	40	41	35	46	44	43	50	55	48	50	56	51	52	60	53	56	66	58
Boots	20	27	20	31	33	31	37	50	39	40	41	42	46	46	48	52	52	52
Cox	24	24	24	30	32	33	33	38	39	35	40	40	39	42	41	41	46	44
Older Lanoxin	30	28	24	38	35	38	43	42	42	50	49	45	57	53	52	57	53	53

digoxin concentrations. The data from the two groups were combined by expressing mean concentrations as percentages of the mean concentration obtained with newer Lanoxin, which had been used by both groups. A good correlation was found at each of the dissolution test time intervals (Table 13). The highest r value was at 30 minutes : $r = 0.89$ ($p < 0.01$). The relationship between the mean digoxin level and percentage of stated dose in solution is illustrated in Figure 11.

The dissolution rate of the batch of Cox brand tablets used in the trial appeared to be much faster than the batch from the same manufacturers reported in Table 7. The dissolution rate of the initial batch had been measured by A.C. Caws at the Burroughs Wellcome laboratory. To assess if these differences reflected any influence from the methodology of the test, the first batch of the Cox brand had its dissolution rate measured by K. Raymond. The results are shown in Figure 12. Raymond also carried out dissolution rate tests on a batch of Lanoxin tablets manufactured prior to the 1969 manufacturing modification. The results of the three types of Lanoxin tablets are shown in Figure 13.

Thirteen patients in each group had atrial fibrillation. The ventricular rates and plasma digoxin concentrations of the patients in group 1 are given in Table 15 and the results of the group 2 patients are in Table 16. The results are summarised in Table 17. The differences in ventricular rate in both groups were statistically significant (analysis of variance, $p < 0.01$).

No patient developed evidence of digitalis toxicity.

In view of the hypothesis from the earlier experience that some patients were consistently more sensitive to changes in tablet brand than others, it was decided to examine if the data gathered in this

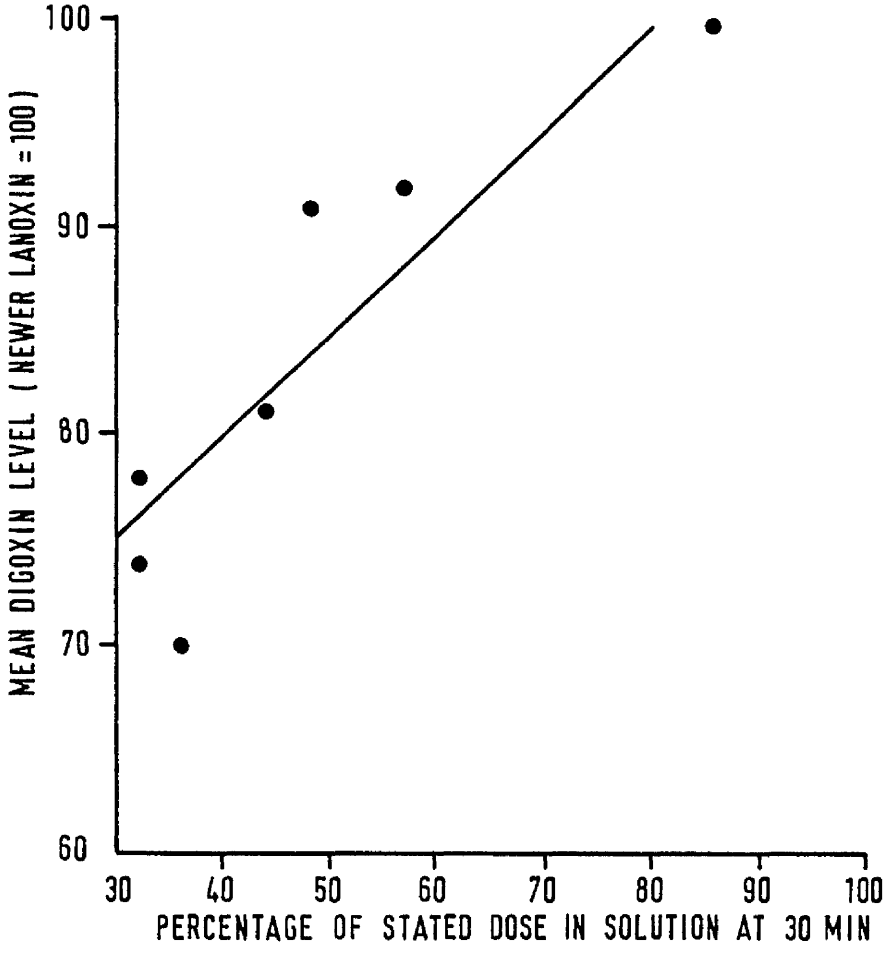

Figure 11 Relationship between percentage dissolution at 30 minutes and plasma digoxin concentration. Mean digoxin concentration obtained with newer Lanoxin = 100, and concentrations found with other brands expressed as a proportion of this figure. $y = 59.3 + 0.5x$.

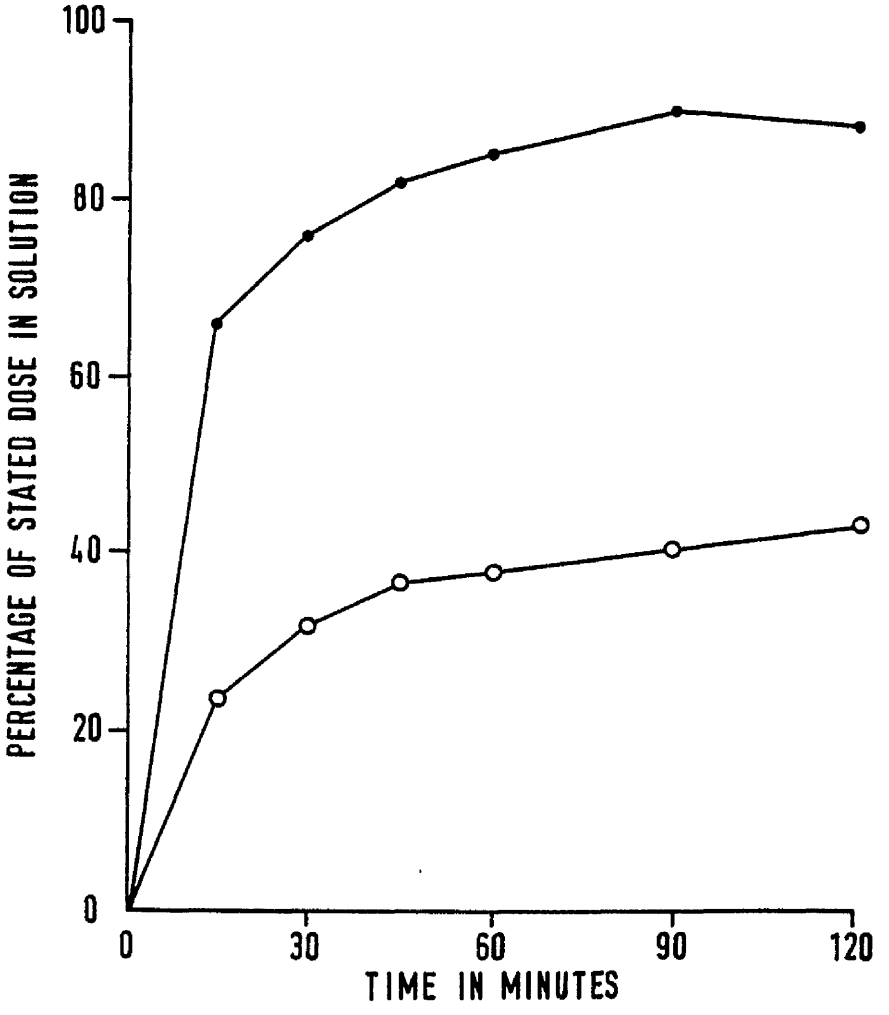

Figure 12 Dissolution rates of two batches of Cox brand digoxin tablets (open circles = batch 212051 - mean of 3 determinations; closed circles = batch 202005 - mean of 2 determinations).

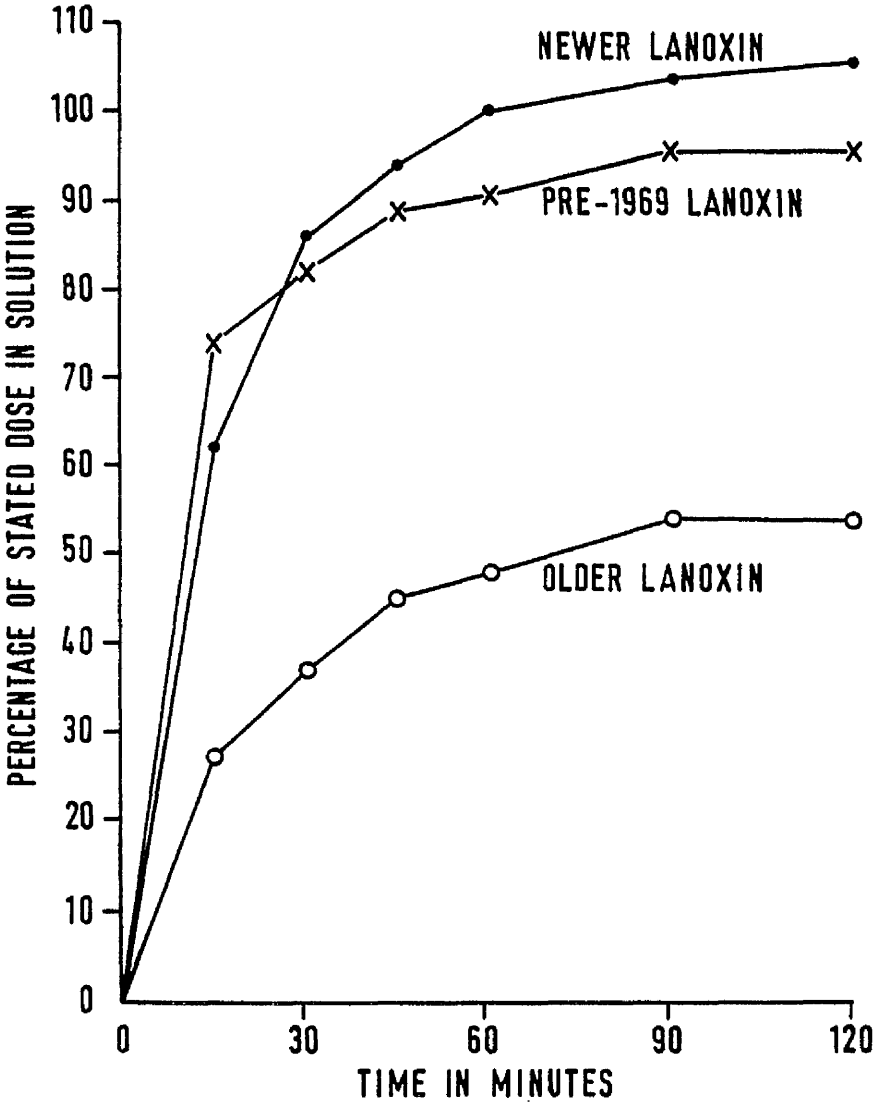

Figure 13 Dissolution rates of three Lanoxin formulations (mean of 3 determinations).

TABLE 15

PLASMA DIGOXIN CONCENTRATIONS (PDC) AND RESTING VENTRICULAR RATE (RVR) OF THE 13 PATIENTS OF GROUP 1 WHO HAD CHRONIC ATRIAL FIBRILLATION

GROUP 1

Patient	Newer Lanoxin		Older Lanoxin		Boots		Nativelle	
	PDC	RVR	PDC	RVR	PDC	RVR	PDC	RVR
2	1.0	110	0.5	106	0.8	102	1.0	108
3	1.3	66	1.1	76	1.0	78	1.5	70
4	1.9	80	1.4	110	1.6	84	2.3	88
7	1.9	70	1.0	70	1.2	70	1.3	82
9	0.7	94	0.6	114	0.7	106	0.8	92
10	1.2	76	0.8	74	0.9	104	0.9	76
11	1.2	68	1.0	62	0.9	76	1.4	64
12	1.0	58	0.8	86	0.9	64	0.8	64
13	2.6	92	2.2	102	1.9	102	2.1	100
14	1.5	68	1.1	82	1.3	88	1.5	80
15	0.8	86	0.5	94	0.7	92	0.7	92
17	1.8	50	1.4	58	1.8	58	1.5	54
18	0.8	56	0.6	64	0.6	64	0.9	58

TABLE 16

PLASMA DIGOXIN CONCENTRATIONS (PDC) AND RESTING VENTRICULAR RATE (RVR) OF THE 13 PATIENTS OF GROUP 2 WHO HAD CHRONIC ATRIAL FIBRILLATION

GROUP 2

Patient	Newer Lanoxin		Cox		Oppenheimer		Macarthys	
	PDC	RVR	PDC	RVR	PDC	RVR	PDC	RVR
1	0.7	132	0.7	112	0.7	92	0.4	94
4	1.8	58	0.5	62	0.8	78	1.5	54
5	1.5	70	1.4	96	1.1	102	1.3	78
6	1.2	86	1.2	92	1.4	80	1.4	80
7	1.0	98	0.7	110	0.6	146	0.6	126
9	0.7	78	0.8	86	0.7	80	1.1	78
11	1.1	88	0.9	112	1.0	90	1.0	88
12	1.3	88	1.1	96	1.2	104	1.3	96
13	0.9	90	0.8	100	0.8	96	1.0	92
15	1.1	72	0.8	82	1.0	64	0.7	68
16	1.0	68	0.6	78	0.6	82	0.7	80
17	1.1	72	0.6	78	0.7	72	0.8	68
19	0.6	70	0.5	58	0.6	78	0.5	60

TABLE 17

RESTING VENTRICULAR RATES AND PLASMA DIGOXIN CONCENTRATIONS (MEANS ± S.D.)
IN PATIENTS OF GROUP 1 AND GROUP 2 WHO HAD CHRONIC ATRIAL FIBRILLATION

GROUP 1 (n = 13)	Newer Lanoxin	Nativelle	Boots	Older Lanoxin
Ventricular rate (beats/min)	74.9 ± 17.1	79.1 ± 16.6	83.7 ± 16.8	84.5 ± 19.2
Plasma digoxin concentration (ng/ml)	1.36 ± 0.56	1.28 ± 0.50	1.10 ± 0.43	1.00 ± 0.47

GROUP 2 (n = 13)	Newer Lanoxin	Macarthys	Oppenheimer	Cox
Ventricular rate (beats/min)	82.3 ± 18.7	81.7 ± 26.4	89.5 ± 20.5	89.4 ± 17.6
Plasma digoxin concentration (ng/ml)	1.08 ± 0.33	0.94 ± 0.36	0.86 ± 0.26	0.81 ± 0.27

trial showed such a pattern. This was done in the following way. Patients were divided into two sections - those who had (1) low or (2) high plasma digoxin concentrations relative to their dose when using the brand of lowest bioavailability in their group. 0.5ng/ml or less was taken to be low for a dose of 0.25mg/day and 1.0ng/ml or less was taken to be low for 0.5mg/day. Since renal impairment would increase the steady state digoxin level for any given dose, patients with a blood urea above 40mg/100ml were excluded. The percentage increase in plasma digoxin concentration after changing from the brand of lowest bioavailability to newer Lanoxin was then calculated (Tables 18 and 19). Those who had low digoxin concentrations relative to dose on the brand of least bioavailability showed a mean increase of 91.0% (S.D. 80.9%) when using newer Lanoxin, while patients with a high concentration showed a smaller increase of 28.5% (S.D. 27.3%). This difference in increase was statistically significant ($p = < 0.05$, unpaired t test). This result did not however prove that a true trend in sensitivity existed, since random error in the radioimmunoassay was bound to have played some part in determining whether the patient was selected for the 'low' or 'high' grouping, and these random errors would not necessarily be replicated at assay of samples taken when the patient used newer Lanoxin. To overcome this effect, the increase in digoxin concentration when changing from the second least bioavailable brand to newer Lanoxin was also calculated, but with the patients remaining in the same 'low' and 'high' groupings which had been made on the basis of the least available brand results. Those in the 'low' group now showed an increase of 50.0% (S.D. 39.1%) while patients in the 'high' group showed a smaller increase of 18.2% (S.D. 21.9%). This difference did not reach statistical significance but the presence of the same trend

TABLE 18

INCREASES IN PLASMA DIGOXIN CONCENTRATIONS (PDC) WHEN PATIENTS WITH LOW PLASMA DIGOXIN CONCENTRATION RELATIVE TO DOSAGE CHANGED (A) FROM THE BRAND OF LEAST BIOAVAILABILITY TO NEWER LANOXIN AND (B) FROM THE BRAND OF SECOND-LOWEST BIOAVAILABILITY TO NEWER LANOXIN

GROUP 1 Patient	Dose (mg/day)	PDC on brand at least bioavailability - older Lanoxin	PDC on newer Lanoxin	% increase	PDC on brand of second lowest bioavailability - Boots	% increase on changing to newer Lanoxin
6	0.25	0.3	0.6	100	0.4	50
15	0.25	0.5	0.8	60	0.7	13
16	0.25	0.4	1.3	225	0.6	117
5	0.5	0.6	1.2	100	0.9	33
7	0.5	1.0	1.9	90	1.2	58
10	0.5	0.8	1.2	50	0.9	33

GROUP 2 Patient	Dose (mg/day)	PDC on brand at least bioavailability - Cox	PDC on newer Lanoxin	% increase	PDC on brand of second lowest bioavailability - Oppenheimer	% increase on changing to newer Lanoxin
2	0.25	0.4	0.6	50	0.4	50
10	0.25	0.5	0.6	20	0.4	50
4	0.5	0.5	1.8	260	0.8	125
13	0.5	0.8	0.9	13	0.8	13
14	0.5	0.9	1.2	33	1.1	8
Mean (n = 11)		0.61	1.10	91.0	0.76	50.0
S.D.		0.23	0.46	80.9	0.28	39.1

supported the hypothesis that some patients were more sensitive than others to change in the dissolution rate of their digoxin tablets.

DISCUSSION

This trial confirmed that different brands of digoxin tablet marketed in the United Kingdom produced significant differences in steady state plasma digoxin concentrations in cardiac patients. These differences existed despite each brand meeting the prevailing British Pharmacopoeia (1968) requirements of digoxin content and disintegration time. The differences in plasma digoxin concentration correlated well with the dissolution rate of these brands despite various manufacturing processes and excipient substances being used.

A strong correlation ($r = 0.97$) between dissolution rate and steady state plasma digoxin concentration was found by Johnson et al., (1973) in a study of 5 experimental batches of Lanoxin with dissolution rates ranging from 54 to 98% at 1 hour. A similar close correlation was obtained with batches from 8 American pharmaceutical companies by Lindenbaum et al., (1973). By 1975, 21 studies had shown a relationship between *in vitro* dissolution rate and digoxin bioavailability (Greenblatt et al., 1976). A few reports appeared to show exceptions to this relationship. Klink et al., (1974) found that a tablet with the remarkably slow dissolution rate of 8% at 1 hour gave moderately high digoxin concentrations during the absorption phase and had an equal area-under-the-curve at 48 hours as a digoxin elixir. These authors admitted to anomalies with their digoxin radioimmunoassay and methodological problems may have influenced the results of this study. Ylitalo, Wilen and Lundell (1975) found similar steady state plasma digoxin concentration with a tablet of 58% - 1 hour dissolution rate

and a digoxin solution. One brand studied by Reissell et al., (1977) appeared to have fast dissolution *in vitro* but poor absorption. However the great weight of published (and unpublished) evidence pointed to a useful correlation between *in vitro* dissolution rate and digoxin tablet bioavailability.

In addition to the variations in digoxin concentration, significant differences were found in the resting ventricular rates of the 26 patients with chronic atrial fibrillation (Tables 15, 16 and 17). When this data was published (Shaw et al., 1973) it was the first study in the literature on any drug to show correlations between dissolution rate, plasma drug concentrations and therapeutic effect. Improved control of atrial fibrillation after a change in digoxin tablet brand was also found by Redfors et al., (1973). Systolic time intervals were noted to change with alteration in digoxin tablet brand (Fleckenstein et al., 1974). Although those working to study digoxin bioavailability tried to ensure that digitalis toxicity was not provoked, a number of cases in clinical practice were seen in which a change of brand provoked digitalis toxicity (Redfors et al., (1973), Shaw (1974)). An outbreak of digoxin toxicity was discovered at a hospital in Israel after a local pharmaceutical company altered the bioavailability of its digoxin tablets (Danon et al., 1977).

Although the *in vitro* dissolution rate test now clearly offered a prospect of ensuring consistent digoxin tablet bioavailability, it remained to establish what the limits for new pharmacopoeal standards of dissolution rate should be.

CHAPTER 5

BIOAVAILABILITY OF VERY RAPIDLY DISSOLVING DIGOXIN TABLET FORMULATION

INTRODUCTION

The close correlation between *in vitro* dissolution rate and steady state plasma digoxin concentrations indicated that this test would be a useful predictor of digoxin tablet bioavailability. When the steady state concentration was plotted against dissolution rate there was a suggestion that there might be a rate above which no further increase in digoxin bioavailability would occur (Lindenbaum et al., 1973, Johnston et al., 1973, Shaw et al., 1973). If this was the case this rate could be used as a minimum dissolution rate requirement for pharmacopoeae to ensure equal bioavailability for all brands of digoxin tablet. To test this hypothesis, 5 formulations, designed to provide very rapid dissolution, were obtained for assessment of their bioavailability.

Formulations producing rapid absorption of digoxin give high plasma digoxin concentrations during the absorptive phase. To investigate if high peak concentrations during absorption might produce transient digoxin toxicity a group of 8 cardiac patients had their electrocardiogram recorded before and after a 0.5mg dose of digoxin solution.

METHODS

Formulations

The formulation studied were:-

1. Lanoxin (batch 1579X) of the type produced and marketed since the production change of 1972 - i.e. standard 'newer' Lanoxin.

2. An experimental batch of Lanoxin containing the same excipients as the standard Lanoxin formulation but modified to produce faster dissolution.

3. A rapidly dissolving brand of digoxin available commercially in Scandanavia - Lanacrist tablets (batch YE 192) produced by the A.B. Draco company in Sweden.

4. A tablet formulation of digoxin prepared in the Department of Pharmacy, Chelsea College, London. This was based on the methodology of Monkhouse and Lach (1972) whereby rapid dissolution of poorly soluble drugs is achieved by adsorbing them to an insoluble agent with an extensive surface, thereby increasing the effective surface area of the drug. In this formulation the digoxin was adsorbed onto calcium carbonate (Mohamad, 1973).

5. An experimental batch of digoxin tablet produced by the Nativelle company of France, containing micronised digoxin with a 50% particle size of 5.6μm.

A fluid preparation of digoxin (Lanoxin for injection, lot 80601) was used in the assessments of rate of absorption.

Dissolution rates

Dissolution rate measurements of these formulations were carried out separately in two laboratories using slightly different techniques.

Dissolution rates measured at the Department of Pharmaceutics, The School of Pharmacy, London were based on the 1970 United States Pharmacopoeia method (Raymond et al., 1974), as described in Chapter 4.

Dissolution rates were also measured by Lars Nyberg in the Analytical Laboratory of the Research and Development Department of A.B. Draco of Lund, Sweden. Ten tablets were placed in a basket

rotating at 100 revolutions per minute in 900ml of simulated gastric fluid without pepsin at 37°C. 5ml samples for fluorimetric assay were taken at intervals up to 2 hours. The method has been described in detail by Nyberg et al., (1974b).

Subjects

Absorption curves

Absorption curves were recorded in two groups of subjects.

A) Four normal ambulant subjects received, in randomised order, 0.5mg doses of digoxin solution and formulations 1, 3 and 4. The dose was given with 40ml of water after overnight fasting. At least one week elapsed between administrations.

B) Four cardiac patients, not normally receiving digoxin, who were free of cardiac failure and in a stable cardiac state received 0.5mg doses of digoxin solution and formulations 1 and 5 in a manner similar to that for first group.

Steady state plasma digoxin concentrations

Eleven cardiac out-patients were given one week courses of formulations 1, 2, 3, 4 and 5 with the order randomised. The dosage used was 0.25mg or 0.5mg per day and was unaltered for each individual during the study. Other medications were not changed. The daily digoxin dose was taken each evening. The discrepancy in tablet count was 1%. There was a period of at least 10 hours (mean 13.0 hours) between the last dose and the blood sample taken at the end of each week's course of digoxin treatment. Ten of the patients had a blood urea of less than 40mg/100ml; one patient had a blood urea of 45mg/100ml. The patients in this group had previously been found to have an increase in plasma digoxin concentration of 40% when they changed from older to newer Lanoxin.

Arrhythmia study group

Eight cardiac patients had lead II of their electrocardiogram recorded for 1 hour before and 6 hours after a 0.5mg dose of digoxin solution. Patients selected for this group were on regular digoxin therapy and had ventricular ectopy considered due to their heart disease and not to digoxin toxicity. Each had a steady state plasma digoxin concentration of less than 2ng/ml. These patients fasted overnight prior to the dose of digoxin solution. They remained semi-supine in bed during the recording period. The characteristics of these patients are included in Table 24. Their rhythm was recorded simultaneously on paper at 5mm/sec and on a tape recorder. The total number of ventricular and supraventricular premature beats per 15 minute interval was counted. If the nature of the rhythm was unclear on the paper record, the corresponding portion was taken from the tape recording and re-played onto paper at a speed of 25 or 50mm/sec. Blood samples for plasma digoxin concentration were taken at 30 minute intervals up to 2 hours and then hourly up to 6 hours.

RESULTS

The dissolution rates of the 5 tablet formulations are given in Tables 20 and 21. The dissolution rate profiles, as measured at the laboratory in Sweden, are illustrated in Figure 14. A good correlation existed between the dissolution rate results of the two laboratories; $r = 0.97$ (20 minutes), $r = 0.98$ (30 minutes). The relationship between results from the two laboratories are shown in Figure 15. In the Swedish laboratory the dissolution rate tended

to be consistently slightly higher than the result obtained for the same formulation in London. The mean amount in solution at 30 minutes was 93% in Sweden and 84% in London.

The absorption curves found in the group A and B subjects are shown in Figures 16 and 17. The peak plasma digoxin concentrations and the time intervals from administration of dose to peak concentration are given in Table 22. Formulations 1, 3, 4 and 5 were all rapidly absorbed although peak levels were slightly less than those found after the dose of digoxin in solution. The normal subjects reported transient nausea at 1 - 2 hours after the dose in 6 of the 16 administrations. The patients in group B said they had not experienced any nausea.

The plasma digoxin concentrations recorded in the 11 out-patients who took one week courses of each formulation are given in Table 23. The mean plasma digoxin concentrations found with each brand were very similar and the very small differences were not statistically significant ($p > 0.05$, paired t test).

The total numbers of ectopic beats recorded per hour in the 8 cardiac patients before and after the 0.5mg dose of digoxin solution are shown in Table 24. The plasma digoxin concentrations recorded before the dose and the peak level after the dose are included in the table. The mean peak level was 3.29ng/ml. Only patient 6 had a transient increase in ectopic count immediately after the dose. The frequency of ectopics in this patient was very variable. Inspection of the ectopic count at 15 minute intervals showed there was not a consistent relationship between ectopic count and rise of digoxin concentration. In no case was coupling or runs of ventricular ectopics seen to coincide with peak digoxin levels.

TABLE 20

DISSOLUTION RATES OF FORMULATIONS 1 - 5 AS MEASURED AT THE SCHOOL OF PHARMACY, LONDON

	Percentage of stated dose in solution at:-						
Formulation	2½ min	5 min	10 min	15 min	20 min	25 min	30 min
1	41	61	72	77	83	85	85
2	30	56	84	87	89	89	91
3	86	89	91	91	86	88	88
4	66	80	85	84	87	88	86
5	24	30	46	57	61	62	70

TABLE 21

DISSOLUTION RATES OF FORMULATIONS 1 - 5 AS MEASURED AT THE ANALYTICAL LABORATORY, AB DRACO, SWEDEN

	Percentage of stated dose in solution at:-									
Formulation	3 min	6 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min	120 min
1	38	61	81	84	88	93	98	100	102	102
2	34	69	90	94	97	98	100	102	101	103
3	95	97	97	98	98	98	97	97	97	98
4	81	87	92	95	96	97	99	98	99	100
5	7	18	44	62	72	79	82	85	89	91

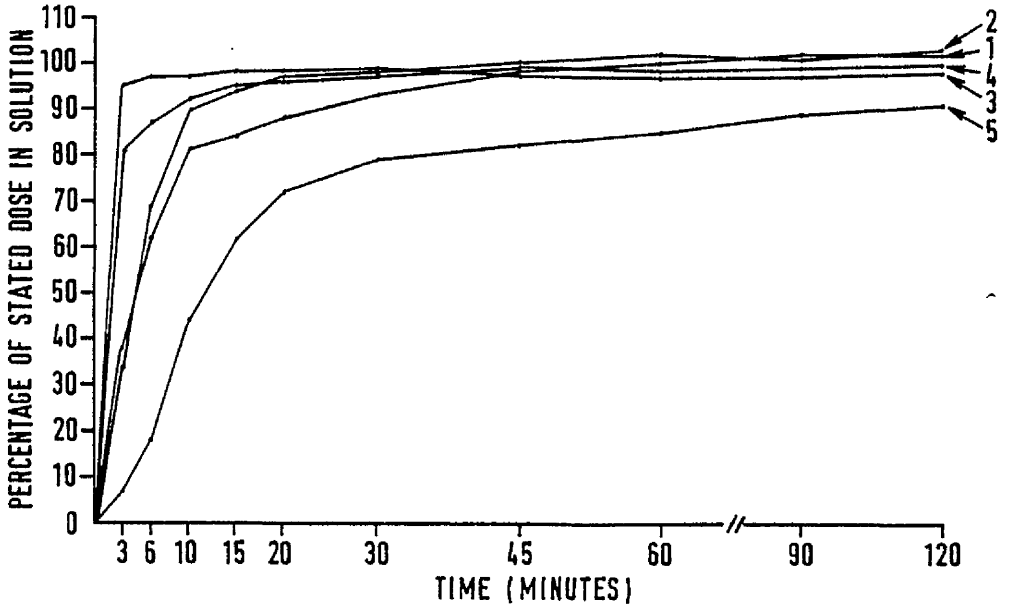

Figure 14 Dissolution rate profiles of formulations 1 - 5 recorded by the Analytical Laboratory, AB Draco. Numbers at right hand side indicate the formulation.

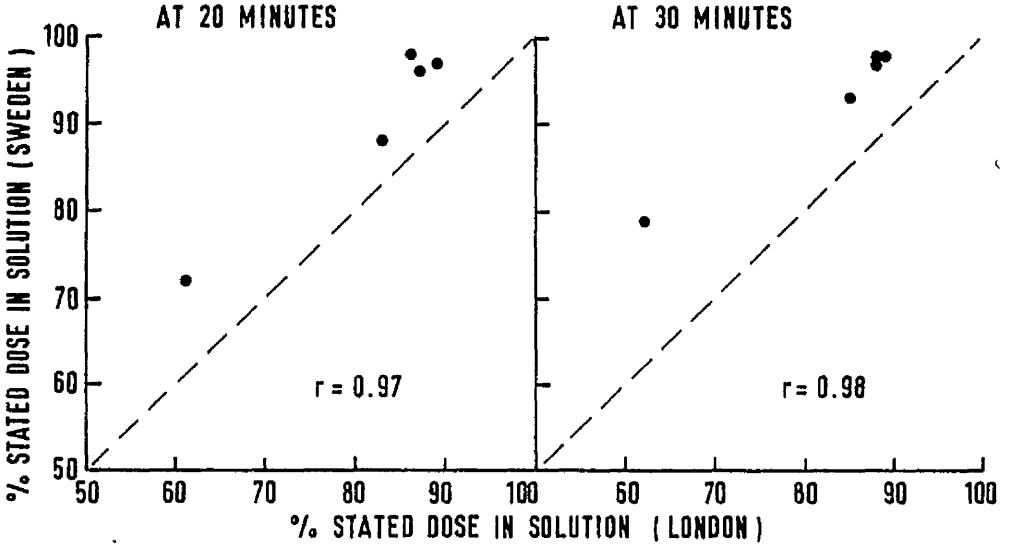

Figure 15 Comparison of dissolution rate results obtained with formulations 1 - 5 in Sweden and London. The broken lines are the lines of identity.

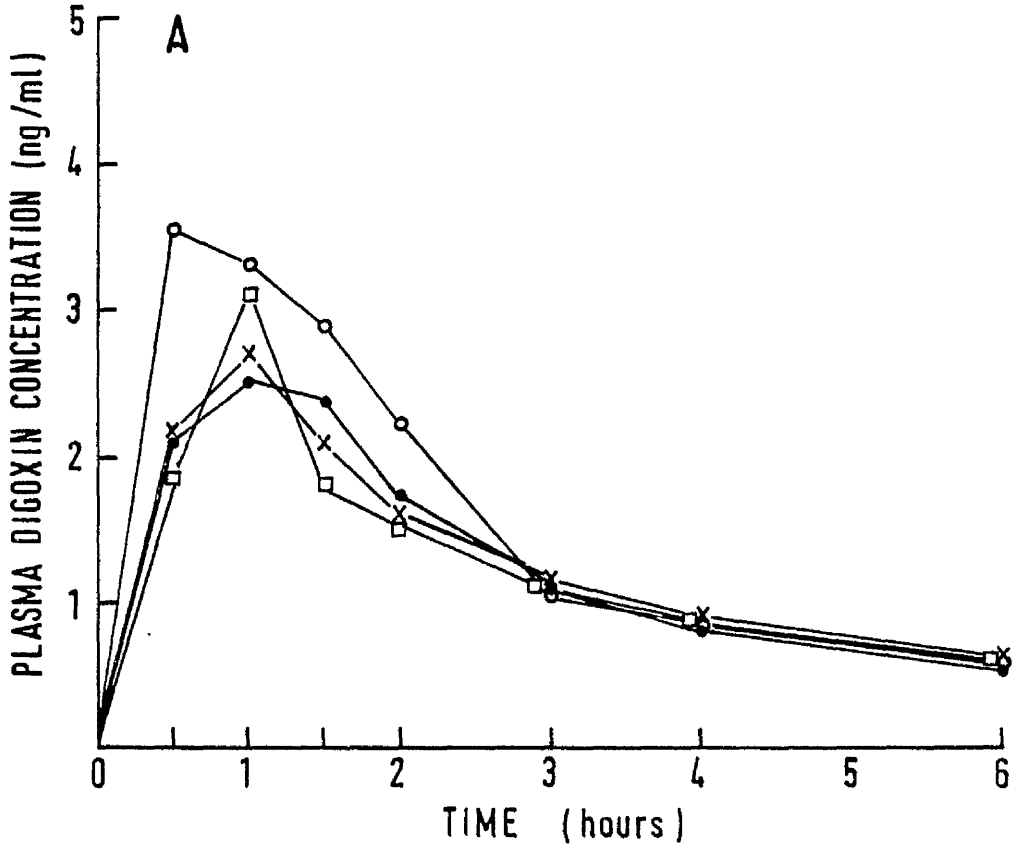

Figure 16 Absorption curves recorded in group A after 0.5mg doses of digoxin solution and formulations 1, 3 and 4. (Open circles - digoxin solution; closed circles - formulation 1; crosses - formulation 3; open squares - formulation 4).

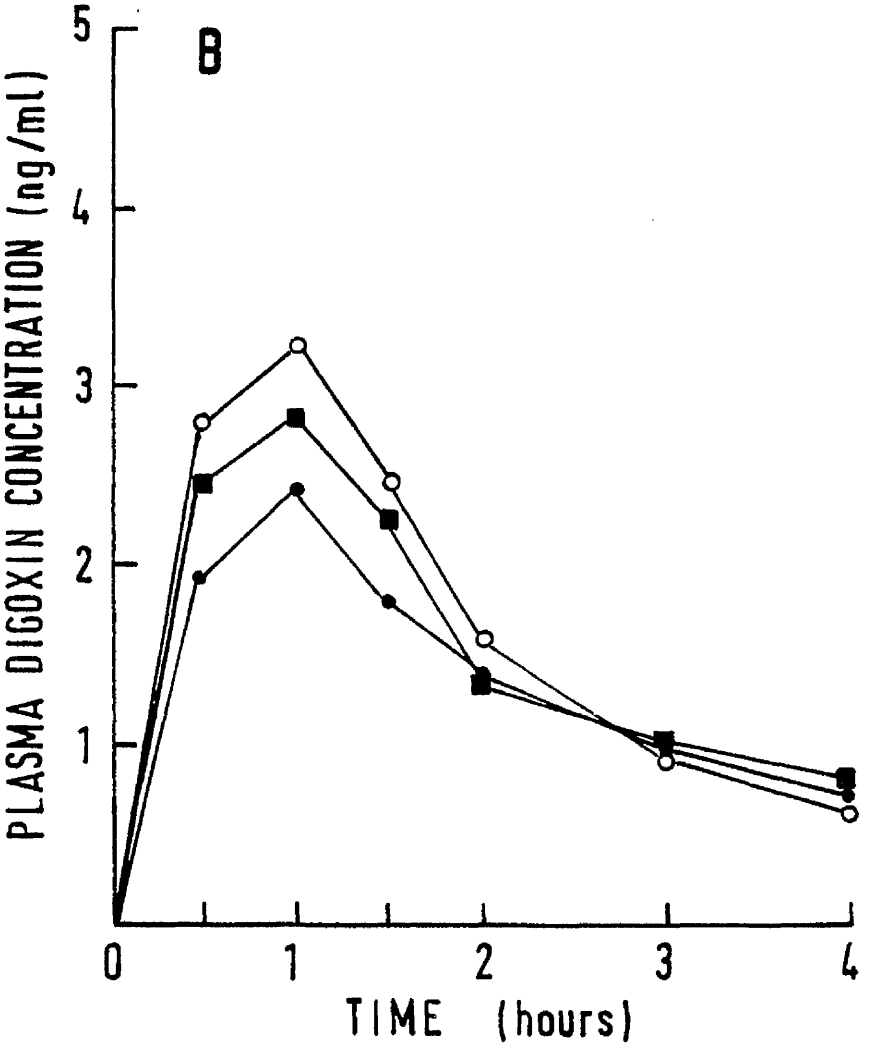

Figure 17 Absorption curves recorded in group B after 0.5mg doses of digoxin solution and formulations 1 and 5. (Open circles - digoxin solution; closed circles - formulation 1; closed squares - formulation 5).

TABLE 22

PEAK PLASMA DIGOXIN CONCENTRATIONS AND TIME INTERVALS BETWEEN DOSE AND PEAK CONCENTRATION IN THE ABSORPTION CURVES OF PATIENTS IN GROUPS A AND B

Formulation	Peak plasma digoxin concentration (ng/ml) Mean ± S.D.	Time interval to peak concentration (minutes) Mean ± S.D.
A) Solution	4.25 ± 0.47	45 ± 30
1	2.75 ± 0.59	60 ± 35
3	3.35 ± 0.86	53 ± 29
4	3.10 ± 0.50	60 ± 0
B) Solution	3.70 ± 1.21	53 ± 15
1	2.73 ± 1.23	60 ± 24
5	3.05 ± 0.61	60 ± 24

TABLE 23

STEADY STATE PLASMA DIGOXIN CONCENTRATIONS FOUND IN 11 PATIENTS AFTER ONE WEEK COURSES OF FORMULATIONS 1 - 5

Formulation	Plasma digoxin concentration (ng/ml)	
	Mean	S.D.
1	1.45	0.33
2	1.47	0.28
3	1.42	0.32
4	1.48	0.50
5	1.45	0.39

TABLE 24

FREQUENCY OF PREMATURE BEATS AND PLASMA DIGOXIN CONCENTRATIONS IN 8 CARDIAC PATIENTS AFTER A 0.5mg DOSE OF DIGOXIN SOLUTION

Patient	Diagnosis	Rhythm		Plasma digoxin concentration		Time of peak PDC (hours)	Number of ectopic beats per hour after dose						
				Pre-dose	Peak		Control	1	2	3	4	5	6
1	AVD	SR	VEB SPB	< 0.25	2.0	½	454	426	421	327	229	-	-
2	AVD/MVD	AF	VEB	0.7	4.5	½	38	17	30	32	24	34	23
3	CM	SR	VEB	1.2	3.4	½	1	0	2	0	4	15	0
4	MVD	AF	VEB	1.2	4.1	2	7	20	14	24	15	12	7
5	IHD	SR	VEB	< 0.25	1.7	½	238	17	85	68	176	224	235
6	AVD	AFL	VEB	0.4	1.9	1	573	945	224	78	116	23	540
7	MVD	SR	SPB	< 0.25	4.3	½	26	4	15	1	1	4	2
8	IHD	SR	VEB	1.1	4.4	½	20	48	58	67	63	59	102

(AVD = aortic valve disease, MVD = mitral valve disease, CM = cardiomyopathy, IHD = ischaemic heart disease, SR = sinus rhythm, AF = atrial fibrillation, AFL = atrial flutter, VEB = ventricular ectopic beat, SPB = supraventricular premature beats).

DISCUSSION

There was a close correlation between the dissolution rate results obtained in London and those obtained in Sweden, although the methods used differed in several characteristics. The methodology of a dissolution rate test could affect the result in a number of ways. These include the tendency to clogging of the mesh basket, the effect of the liquid medium on the tablets and on the metal components of the apparatus, and the mixing pattern produced by vibration and the shape of the vessel (Raymond et al., 1974). Studies undertaken by the British Pharmacopoeia Commission showed that interlaboratory differences in digoxin tablet dissolution rate of reference formulations were not so large as to preclude the setting of dissolution rate criteria (unpublished data).

Each of the tablet formulations were found to have a rapid dissolution rate. The slowest dissolving formulation was formulation 5 which had a rate of 70% in solution at 30 minutes as measured in the London laboratory, and of 79% at 30 minutes, 85% at 60 minutes and 91% at 120 minutes in the Swedish laboratory. As each of the formulations produced similar steady state plasma digoxin concentrations this suggested that a dissolution rate in the range 70 - 80% at 30 minutes would be a suitable standard to ensure equal digoxin tablet bioavailability.

Johnson and Lader (1974) compared the bioavailability of standard newer Lanoxin (dissolution rate 79% at 15 minutes and 98% at 60 minutes) with Lanoxin modified to produce very rapid dissolution and with capsules containing ultra-rapidly dissolving digoxin (both released 100% of the dose in 7.5 minutes). They measured the areas under absorption curves of 80 hours duration and the urinary digoxin

excretion over 10 days. They found no difference between these three formulations. Each had a bioavailability equivalent to a digoxin solution. Greenblatt and Koch Weser (1974) found no difference in bioavailability between tablets of dissolution rates 85% in 1 hour and 90 to 95% in 1 hour. Both provided absorption equivalent to a liquid preparation of digoxin.

When the drug regulating agencies came to devise standards for digoxin bioavailability they adopted different approaches. In Britain the first step had been to advise that Lanoxin should be dispensed only when it had been specified by the prescriber (Lancet, 1972). This followed the unexpected increase in Lanoxin bioavailability and was designed to prevent an outbreak of toxicity. The British Pharmacopoeia added an amendment (effective from 1st February 1973) to its 1968 Edition to establish limits on the content of individual digoxin tablets, whereas previously content had been measured in a combined sample of 20 tablets (Pharmaceutical Journal, 1972b). This was based on published (Oudtshoorn (1972), Manninen and Korhonen (1973)) and unpublished information that some brands of digoxin tablet showed a very wide variation in the content of individual tablets. This action probably removed some brands of low bioavailability from the market since marked variation in content reflects poor mixing during tabletting and this could be associated with low bioavailability (see Chapter 6). British digoxin tablets now had to meet the following content requirements : 9 of 10 tablets tested to be within 80 - 120% of stated dose and all within 75 - 125%. In 1973 the Department of Health and Social Security undertook a review of all product licences issued to pharmaceutical companies in respect of digoxin tablets (Pharmaceutical Journal, 1973). The question of

digoxin bioavailability was reviewed by a Digoxin Tablets Panel of the Medical Chemicals Committee of the British Pharmacopoeia Commission. From 1st October 1975, the Pharmacopoeia had an added amendment requiring all digoxin tablets marketed within the United Kingdom to have a dissolution rate of not less than 75% of stated dose in solution at 1 hour.

Not all countries adopted the same standard. In the Netherlands the dissolution rate limit was set at 90% at 1 hour. The Italian Pharmacopoeia required a rate exceeding 70% at 30 minutes (Stewart 1981). In the United States the Food and Drug Administration had not had the problem of a sudden change in the leading digoxin brand. They were faced however with products whose dissolution rate ranged from 3.8% to 93.5% at 1 hour (Harter, Skelly and Steers, 1974). As a first step they wished to eliminate very poorly absorbed brands, but also to delay the marketing of very rapidly dissolving formulations until the safety of rapid absorption had been re-assessed. Accordingly they set both lower and upper limits for permitted dissolution rate : 55% and 95% at 1 hour. The former was soon afterwards raised to 65% (Harter et al., (1974), Harter (1975)). The digoxin dissolution rate method of the U.S. Pharmacopoeia uses a series of single tablet estimations whereas in the British method a group of 6 tablets are used. The FDA also required from each manufacturer in vivo bioavailability data to show that in 12 subjects the area under the digoxin plasma concentration curve from 0 - 5 hours after single doses is at least 75% of the mean of areas from a digoxin solution and a reference tablet formulation with a dissolution rate of 75% at 1 hour. This protocol has been critised as imprecise (Kramer et al., 1977, Wagner et al., 1977).

The fact that the American standards encompass digoxin tablets of incomplete relative bioavailability has been shown by studies which have compared U.S. Lanoxin with digoxin solution. Greenblatt et al., (1974) found 24% higher bioavailability from tablets of dissolution rate 85 - 90% at 1 hour compared with those of dissolution rate 64 - 65% at 1 hour. U.S. Lanoxin was less well absorbed than a digoxin solution in studies by Greenblatt et al., (1973), Huffman et al., (1974), and Lloyd et al., (1978), although Marcus et al., (1976) found equivalent bioavailability. When the results of these 4 studies are combined digoxin solution has an average bioavailability of 67% relative to an intravenous dose, and U.S. Lanoxin tablets have a bioavailability of 55%. From the biopharmaceutical point of view the submaximal absorption of American digoxin tablets represents a less than ideal situation since inter-subject differences in absorption become more marked as dissolution rate and absorption decrease (Levy and Gibaldi, 1974, Johnson et al., 1976a, Johnson, Smith and French, 1977).

One report suggested that rapid absorption of digoxin, and its associated higher plasma digoxin concentrations, might produce transient digitalis toxicity (Manninen, Reissell and Paukkala, 1976). This study lacked a control period and did not accord with many unpublished observations of others or with the results in the cardiac patients reported in thisChapter. The patients reported here had all shown a tendency to ectopic inpulse formulation but this did not show any tendency to increase along with the rise in digoxin concentration during the absorption phase, although plasma digoxin concentration rose well above 2ng/ml. No transient arrhythmias were seen in a study of cardiac paediatric cases (Larese and Mirkin (1974)). These findings reflect the fact that absorption phase plasma digoxin concentrations are not identical to digoxin receptor concentration.

Nausea was reported in 6 of 16 administrations of the 0.5mg dose by the normal subjects in group A (each of whom was a hospital pharmacist) but no nausea was reported in group B. Nausea had not been reported when the out-patient survey patients were changed to newer Lanoxin, although each patient had been questioned about symptoms of possible toxicity. The basis for the high incidence of early and transient nausea in group A is not clear. In my own experience with rapidly dissolving tablets in clinical practice gastric intolerance of digoxin is seen very occasionally, but is probably more frequent than had been the case with slowly dissolving formulations.

The digoxin bioavailability studies of the 1970's acted as a reminder that the absorption of oral digoxin, even as a solution, was incomplete. Three approaches were used in attempts to find an improvement on this situation. The other naturally-occurring cardiac glycosides were re-assessed, new types of digoxin formulation were developed, and semi-synthetic derivatives of digoxin were evaluated.

Digitoxin is more lipophilic than digoxin and had appeared to be completely absorbed in the studies by Gold et al., (1953) and Weissler et al., (1966). Virtually complete absorption from solution and rapidly dissolving tablets of digitoxin was confirmed in more recent work (Beermann,Hellstrom and Rosen (1971), Vohringer, Wogenstein and Rietbrock (1977), Vohringer, Leopold and Rietbrock (1977), Greeff et al., (1977), Greeff et al., (1979), Storstein and Johsgard (1981)). Stoll et al., (1973) had found similar bioavailability with two commercial brands of digitoxin tablets, one having a dissolution rate of 95% at 15 minutes and the other 65% at 60 minutes. However, Wood et al., (1975) later stated that digitoxin bioavailability showed "a clear dependency"

on dissolution rate. This brief report did not include any data. Much of the information on digitoxin tablet characteristics appears to have remained unpublished in FDA files. The FDA Drug Bulletin (1976) did report that in the FDA tests of commercial digitoxin tablets some had shown "absorption as low as 60%". The magnitude of variation between brands was less than the FDA had found in tests of digoxin tablets (where they had found some brands had a total absorption "as low as 10 - 15%"), however approximately 50% of digitoxin brands had to be reformulated when the dissolution rate criteria was raised to not less than 50% at 30 minutes and not less than 85% at 1 hour. A real but less publicised bioavailability problem did therefore exist with digitoxin. The FDA also stated that it had proved possible to manufacture digitoxin tablets of "100% bioavailability". However inter-subject variation in steady state plasma concentration and pharmacologic effect is as great with digitoxin as with digoxin (Belz et al., 1978), reflecting the importance of other pharmacokinetic and physiological factors.

In 1981 Sandoz Products Ltd. announced that a reformulation of their lanatoside C (Cedilanid) tablets had given "greater bioavailability". Some novel digoxin formulations appeared. A digoxin inert carrier precipitate (a concept similar to formulation 4) was assessed in vitro and gave rapid dissolution rates (Reddy, Khalil and Gouda (1976)). Bochner et al., (1977) developed a digoxin-hydroquinone complex formulation which gave 99% dissolution at 5 minutes. However, no greater absorption was found than with conventional tablets of dissolution rate 74% at 30 minutes and 88% at 60 minutes. The greatest interest was shown in a special liquid digoxin preparation which was encased in a soft gelatin capsule. The digoxin was dissolved in a polyethylene glycol-ethanol solution and this, rather than the encapsulation, appeared to have an influence on absorption (O'Grady et al., 1978).

A number of investigators reported on this type of formulation - Mallis et al., (1975), Johnson et al., (1976a), Binnion (1976), Marcus et al., (1976), Lindenbaum (1977) and Lloyd et al., (1978). Overall in these studies there was a mean increase of 17% in absorption relative to solution when absorption was assessed by the area-under-the-curve method, and of 12% when absorptions were compared using cumulative urinary excretion. Absolute bioavailability was still sub-maximal, averaging 83% when results of the plasma and urinary indices were combined. Most of these studies did not show a significant decrease in inter-subject variability, except when comparison was with U.S. Lanoxin tablets rather than solution. Rodgers et al., (1977) did not find any improvement in clinical effect when capsules were used instead of British newer Lanoxin tablets. Considerations of cost-effectiveness appear to have inhibited these newer formulations from achieving commercial production.

Methyl digoxin is a semi-synthetic derivative of digoxin which has a methyl group attached to the terminal sugar, giving it greater water and lipid solubility than digoxin and resulting in greater hepatic metabolism. Single dose studies (Boerner et al., (1976), Rietbrock et al., (1976), Hinderling et al., (1977)) indicated that methyl digoxin had a high but still incomplete bioavailability. Greeff et al., (1977) found it to be only slightly better absorbed than digoxin. Similar results have been obtained with acetyl digoxin. The slight improvement in absorption does not seem to have outweighed the greater familiarity which clinicians have with digoxin, and digoxin remained the most commonly prescribed cardiac glycoside.

CHAPTER 6

THE EFFECT OF PARTICLE SIZE ON THE ABSORPTION OF DIGOXIN

INTRODUCTION

While the dissolution rate test offered an effective method for ensuring equivalent digoxin tablet formations from different manufacturers, it was important for those producing digoxin tablets to be aware of the parameters which determined digoxin bioavailability. Only in this way could they design their production process with confidence and prevent minor alterations in technique from altering batch to batch consistency.

After discussions with many pharmaceutical companies, I could not find any excipient substance which would explain differences in dissolution. All of the British companies who made digoxin tablets began with powder of pure digoxin supplied from one of three sources - Burroughs Wellcome Ltd, Wander Ltd and the Nativelle company of France - and yet ended with formulations of very differing dissolution rates. When a drug is poorly soluble in water the major determinant of its release into solution, given a rapid tablet disintegration rate, is the particle size of the drug in the tablet. A change in particle size as the explanation for alterations in digoxin bioavailability fitted with the early observation that grinding a poorly absorbed tablet into a fine powder increased absorption. This concept was also supported by the knowledge that manufacturers used different trituration and grinding times in their tabletting process, and this stage of tabletting is known to be able to alter particle size (Cooper and Rees, 1972).

A difficulty in testing the hypothesis that particle size influenced digoxin absorption was that if a powder of known particle size was made into a tablet in the normal way, the final particle size became unknown. It was decided to circumvent this problem by using cachets to enclose digoxin powders of known particle size. It was decided to study the absorption obtained with the unmodified digoxin powder as supplied to pharmaceutical companies and then to study how reduction in the particle size of this powder influenced absorption.

METHODS

Digoxin powders

Three digoxin powders used commercially in the United Kingdom for digoxin tablet production were obtained for measurement of particle size. These powders were from (1) Burrough Wellcome Ltd, batch 3608, (2) Laboratoire Nativelle (Paris), batch 4500 VIII 1972 and (3) Wander Ltd (a British distributor of Sandoz), batch 10702.

To produce a powder of smaller particle size a 250mg portion of the Burrough Wellcome powder was milled in a Glen Greston M270 agate vibratory ball mill for 15 minutes.

A second portion of the same powder was similarly milled for 45 minutes. With this duration of milling it was found necessary to add as lubricant 2% Aerosol 200 (Degussa) to prevent caking of the powder.

Melting point behaviour of the digoxin, recorded with a Perkin Elmer Differential Scanning Calorimeter, did not alter after the 15 or 45 minute milling, suggesting that the milling had not altered the digoxin crystalline form.

Measurement of particle size

The particle sizes of the Burroughs Wellcome, Nativelle and Wander powders were determined with a photoextinction sedimentometer (Evans Electroselenium Ltd). The powder was suspended in water which contained a non-ionic wetting agent and was dispersed by agitation in an ultrasonic bath. Particle size distribution was calculated by the method of Edmundson (1967).

Particle size of the ball milled samples was measured by Coulter Counter (Model B : Coulter Electronics Inc.) fitted with a 100μm orifice tube. The digoxin was suspended in 0.9% NaCl solution containing a non-ionic wetting agent in an ultrasonic bath.

Preparation of cachets

Sets of rice paper cachets containing the unmilled Burroughs Wellcome powder, the 15 minute milled powder and the 45 minute milled powder were prepared. Each type of powder was first mixed by trituration with lactose in a small mortar. The trituration process was standardised to avoid further reduction of digoxin particle size. Each cachet contained 0.25mg or 0.5mg digoxin and lactose to a total weight of 200mg. Digoxin content of individual cachets was measured and did not exceed ±15% of nominal dose.

The measurement of particle size and preparation of the cachets were carried out by Professor J.E. Carless of the Department of Pharmacy, Chelsea College, London.

Cachet dissolution rate tests

The cachets were examined by K. Raymond with the in vitro dissolution rate test apparatus described in chapter 4. Single cachets were used.

Patients

Group A

The rate of digoxin absorption from the unmilled Burroughs Wellcome powder and the 45 minute milled powder were compared with that from a digoxin solution (Lanoxin for injection) in 4 normal ambulant subjects. A single cachet of 0.5mg digoxin was taken with 40ml water after an overnight fast. The order of administration was randomised. There was a one week interval between doses. Plasma digoxin concentrations were recorded for 6 hours after the dose.

Group B

The unmilled powder was also compared with the 15 minute milled powder in 4 ambulant cardiac patients who were on regular digoxin treatment. A 0.5mg digoxin cachet was administered in place of the patients' normal tablet dose. The patients had fasted overnight. Blood samples were taken before the dose and for 3 hours after.

Group C

The unmilled powder was compared with the 45 minute milled powder in a second group of 4 patients in the same manner to that used with group B.

Group D

The extent of digoxin absorption from each type of cachet was assessed by measurement of steady state plasma digoxin concentrations in 9 cardiac out-patients. The unmilled and the 15 minute milled powders were given in randomised order. The 45 minute milled powder cachets became available later and were administered 2 - 4 weeks after the first two sets of cachets. Digoxin dosage was kept constant and other medication was not changed. A blood sample for plasma digoxin concentration estimation was taken after one week of treatment

of each type of cachet. The sample was taken 6 - 12 hours after the last dose, with the interval kept constant for each individual. The personal characteristics of these patients are included in Table 28.

RESULTS

The particle diameters of the Burroughs Wellcome, Nativelle and Wander powders were found, in each instance, to be distributed in a log-normal fashion i.e. there was a normal (bell) shape of distribution when particle diameter was plotted on a logarithmic scale. With this type of distribution the arithmetic mean is not appropriate and is replaced by calculation of the "50% size" i.e. the diameter size above and below which 50% of the measured diameters are distributed. The 50% sizes of the 3 commercial digoxin powders are given in Table 25. The geometric standard deviations and the surface areas of the powders were calculated from the log-probability plots of particle diameter (Thornton,1959, Edmundson, 1967) and are given in Table 25. The Burroughs Wellcome and Nativelle powders had a very similar pattern of particle size. The sizes in the Wander powder were slightly larger. The Nativelle powder had a surface area 39% larger than that of the Wander powder.

Ball-milling of the Burroughs Wellcome powder did not alter the log-normal distribution pattern. The 50% sizes and surface areas of the milled powders are included in Table 25. The distribution of particle sizes are shown on a log-probability plot of particle diameter in Figure 18. The 50% size of the 45 minute milled powder has to be regarded as approximate and as an overestimate of the true particle size as the distribution of sizes of this powder would suggest that a number of particles would be below the limit of detection (2μm) of the measurement technique.

TABLE 25

PARTICLE SIZE AND SURFACE AREA OF DIGOXIN POWDERS

Powder	50% size (μm)	Geometric standard deviation (μm)	Surface area (m^2/g)
Burroughs Wellcome	22	1.96	0.23
Nativelle	20	2.00	0.25
Wander	29	2.16	0.18
Burroughs Wellcome - milled 15 min	12	2.00	0.42
Burroughs Wellcome - milled 45 min	3.7	2.35	1.56

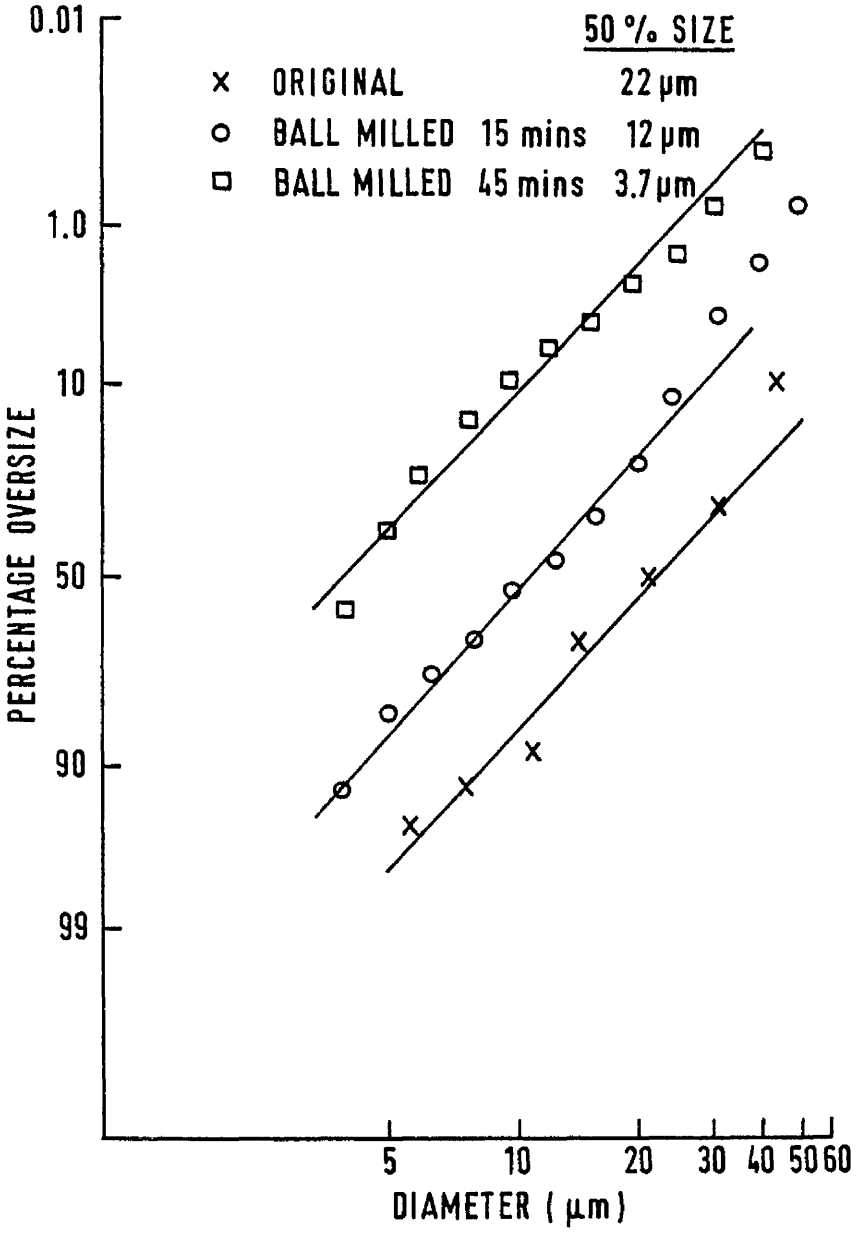

Figure 18 Log-probability plot of particle size of Burroughs Wellcome digoxin powder before and after ball-milling.

The absorption curves of digoxin from the unmilled Burroughs Wellcome powder, from the 45 minute milled powder and from the digoxin solution, as recorded in group A, are illustrated in Figure 19. The peak plasma digoxin concentrations and the times to the peak are given in Table 26. The peak concentrations found with the 45 minute milled powder were significantly higher than those recorded after the unmilled dose ($P < 0.05$, paired t test). Both cachet formulations were more slowly absorbed than the solution.

The absorption curves recorded in the groups B and C are shown in Figure 20. The peak rise in digoxin level and the times to the peak rise are given in Table 27. Administration of both milled powders showed a faster absorption profile than was recorded after the unmilled powder dose. With the 45 minute milled powder peak digoxin concentrations were higher and earlier than those after the 15 minute milled powder in the other group.

Plasma digoxin concentrations found in the patients of group D at the end of each week's treatment with cachets of unmilled powder, 15 minute milled powder, and 45 minute milled powder are given in Table 28 and are shown in Figure 21. Patient 3 developed persistent nausea on the sixth day of treatment with the 45 minute milled powder cachets: she omitted her last dose and a plasma digoxin concentration on this formulation was not recorded. With this case excluded there was a 31% rise in mean steady state digoxin concentration from 0.80 S.D. 0.21ng/ml to 1.05 S.D. 0.35ng/ml between use of powders of 22μm (unmilled) and 3.7μm (45 minute milled) particle size, ($p < 0.01$, paired t test). If a digoxin level of 2ng/ml is assumed for patient 3, the increase in digoxin level was 45%.

The amounts of digoxin in solution at 60 minutes in the *in vitro* dissolution rate tests were: unmilled 9%, 15 minute milled 18%;

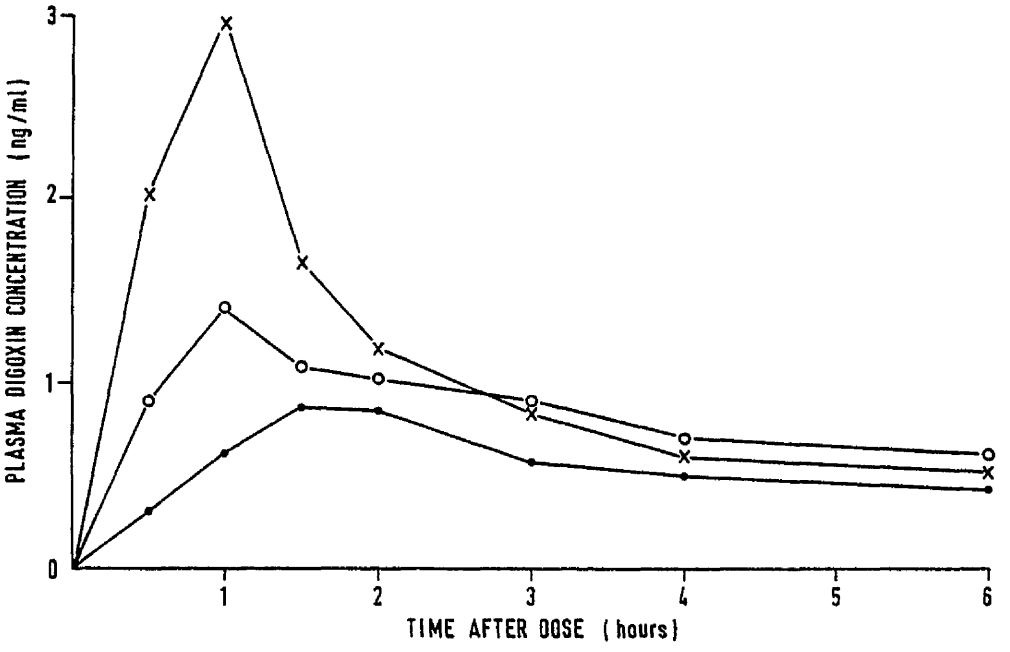

Figure 19 Absorption curves recorded in 4 normal subjects (Group A) after 0.5mg digoxin doses administered as unmilled digoxin powder in a cachet (closed circles), 45 minute milled digoxin powder in a cachet (open circles) and a solution of digoxin (crosses).

TABLE 26

PEAK PLASMA DIGOXIN CONCENTRATIONS (PDC) IN ng/ml
AND TIME TO PEAK CONCENTRATION IN 4 NORMAL SUBJECTS
AFTER 0.5mg DOSES OF DIGOXIN POWDER
IN CACHETS AND AFTER 0.5mg OF DIGOXIN SOLUTION

Formulation	Peak PDC Mean ± S.D. (range)	Time (hours) to peak PDC Mean ± S.D. (range)
Unmilled powder (cachet)	0.93 ± 0.05 (0.9 - 1.0)	1.63 ± 0.25 (1.5 - 2.0)
45 minute milled powder (cachet)	1.93 ± 0.53 (0.9 - 2.1)	1.00 ± 0.41 (0.5 - 1.5)
solution	3.25 ± 1.59 (2.0 - 5.4)	0.75 ± 0.29 (0.5 - 1.0)

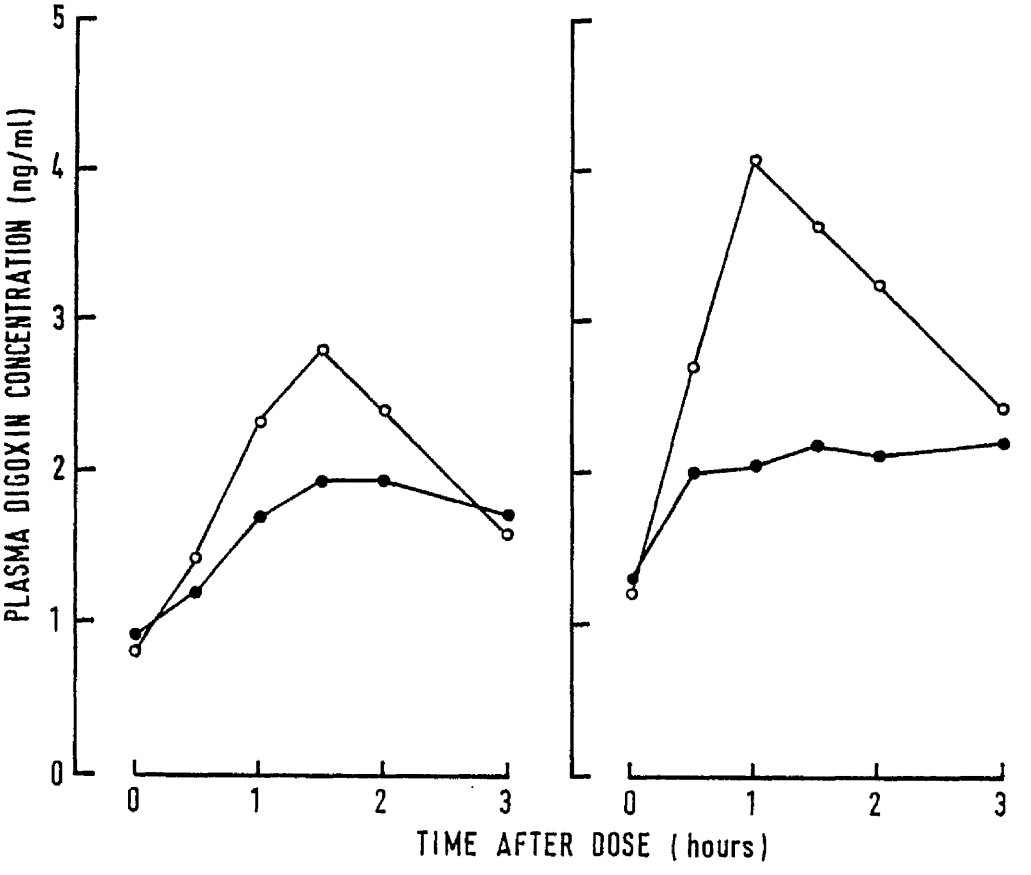

Figure 20 Absorption curves recorded in Group B (left hand panel) and Group C (right hand panel). Group B received 0.5mg doses of unmilled powder (closed circles) and 15 minute milled powder (open circles). Group C received 0.5mg doses of unmilled powder (closed circles) and 45 minute milled powder (open circles).

TABLE 27

PEAK RISE IN PLASMA DIGOXIN CONCENTRATIONS (PDC) AND TIME TO PEAK RISE IN GROUPS B ANC C AFTER 0.5mg DOSES OF DIGOXIN POWDER FORMULATIONS

Group	Formulation	Peak rise (ng/ml) in PDC Mean ± S.D. (range)	Time (hours) to peak PDC Mean ± S.D. (range)
B	Unmilled powder	1.27 ± 0.59 (0.8 - 2.1)	1.87 ± 0.85 (1.0 - 3.0)
	15 minute milled powder	2.00 ± 0.93 (1.1 - 3.3)	1.38 ± 0.25 (1.0 - 1.5)
C	Unmilled powder	1.10 ± 0.28 (0.7 - 1.3)	1.88 ± 0.85 (1.0 - 3.0)
	45 minute milled powder	2.98 ± 1.14 (1.6 - 4.4)	1.13 ± 0.25 (1.0 - 1.5)

TABLE 28

PERSONAL CHARACTERISTICS AND PLASMA DIGOXIN CONCENTRATIONS OF 9 PATIENTS USING DIGOXIN CACHETS CONTAINING POWDER OF DIFFERING PARTICLE SIZE

Patient	Weight (Kg)	Height (cm)	Blood urea (mg/100ml)	Digoxin dose (mg/day)	Plasma digoxin concentration (ng/ml)		
					Unmilled powder	15 min milled powder	45 min milled powder
1	85	180	39	0.25	0.5	0.4	0.5
2	63	162	26	0.25	0.5	0.6	0.7
3	60	160	37	0.25	0.6	1.5	TOXIC
4	57	158	54	0.25	0.7	0.7	0.8
5	81	179	23	0.5	0.8	0.8	1.2
6	64	170	26	0.5	0.9	1.3	1.5
7	76	182	31	0.5	1.0	1.1	1.3
8	68	174	28	0.5	1.0	1.1	1.3
9	93	183	36	0.5	1.0	0.9	1.1

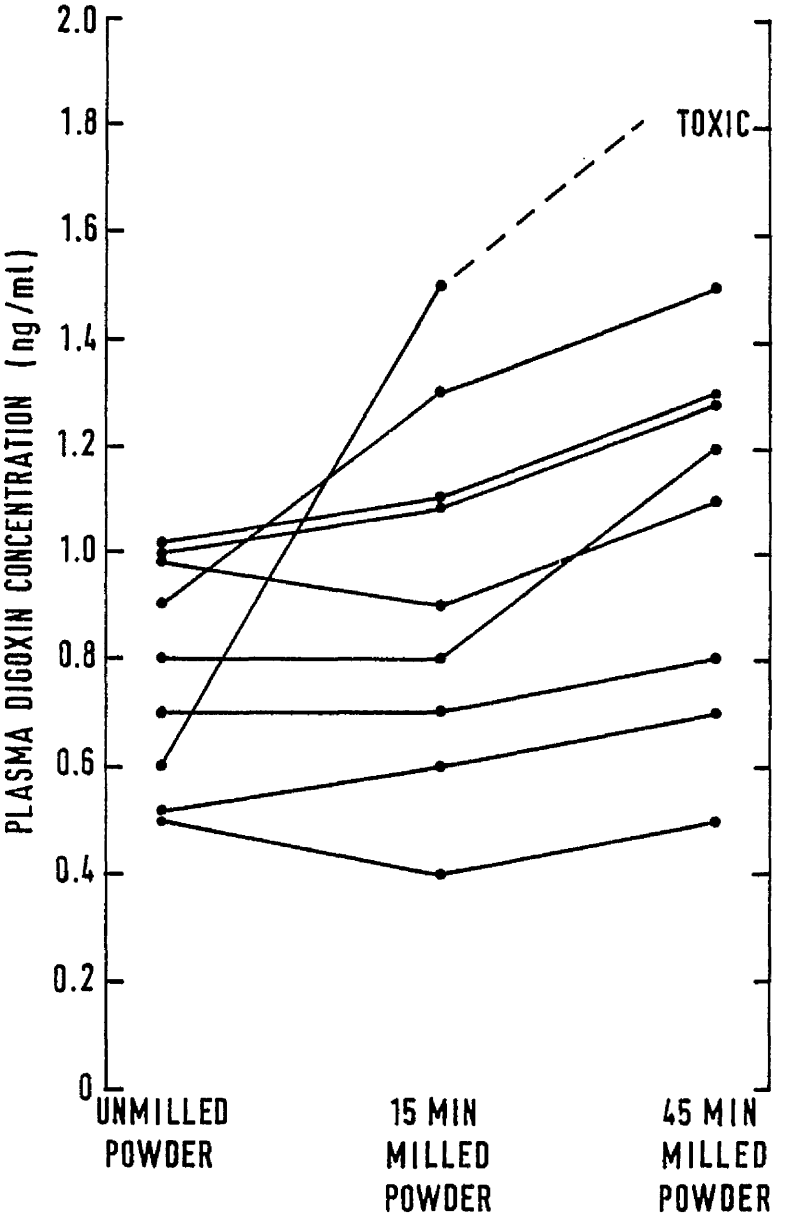

Figure 21 Steady state plasma digoxin concentrations recorded in 9 out-patients after each weeks' treatment with cachets containing digoxin powder of differing particle size.

45 minute milled 25% (Figure 22). The cachets did not disintegrate although they did become permeated with water (Figure 23).

DISCUSSION

These results showed that the 3 commercially-available digoxin powders used for digoxin tabletting in the United Kingdom all had particle diameters whose 50% size was in the range 20 - 30μm. When the unmilled Burroughs Wellcome powder was mixed with only lactose and administered within a cachet the absorption of digoxin was slower and less complete than that obtained with powders milled to give particle sizes of 12 and 3.7μm.

In vitro dissolution rate also increased as particle size reduced. The ranking in this test would be valid but the absolute values of dissolution rate must have been affected by the lack of disintegration of the cachet walls. This would prevent circulation of the water within the cachet. The effect of particle size on the dissolution rate of digoxin powders was also demonstrated by Florence, Salole and Stenlake (1974) who also found evidence to suggest that dry grinding altered the proportion of amorphous and crystalline forms. Johnson, O'Grady and Bye (1978) obtained a dissolution rate of 100% at 7.5 minutes with tablets containing digoxin of particle size "under 10μm" and a rate of 34% at 1 hour with tablets of digoxin of particle size "90 - 106μm".

Other workers have confirmed the important influence of particle size on digoxin absorption. Jounela, Pentikainen and Sothman (1975) recorded area-under-the-curve and urinary digoxin excretion indices at steady state in 7 subjects. Tablets of digoxin of mean diameters (sic) 13 and 7μm gave equivalent absorption to

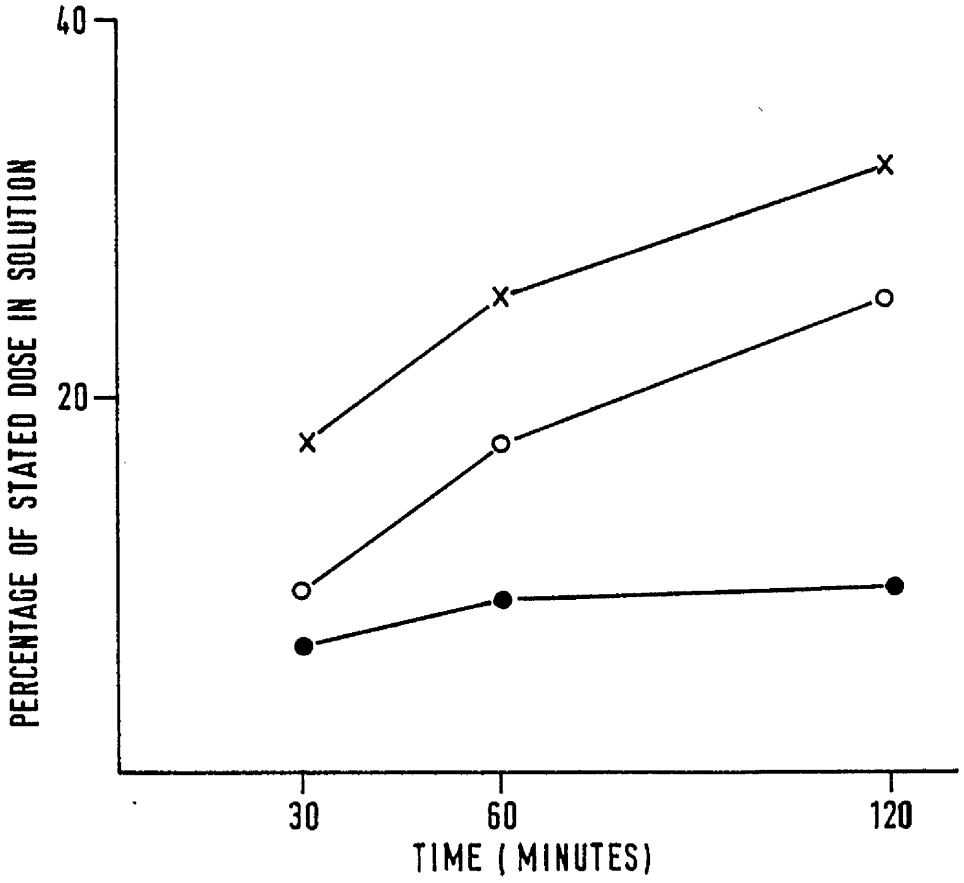

Figure 22 Dissolution rate profiles of cachets containing unmilled digoxin powder (closed circles), 15 minute milled powder (open circles) and 45 minute milled powder (crosses).

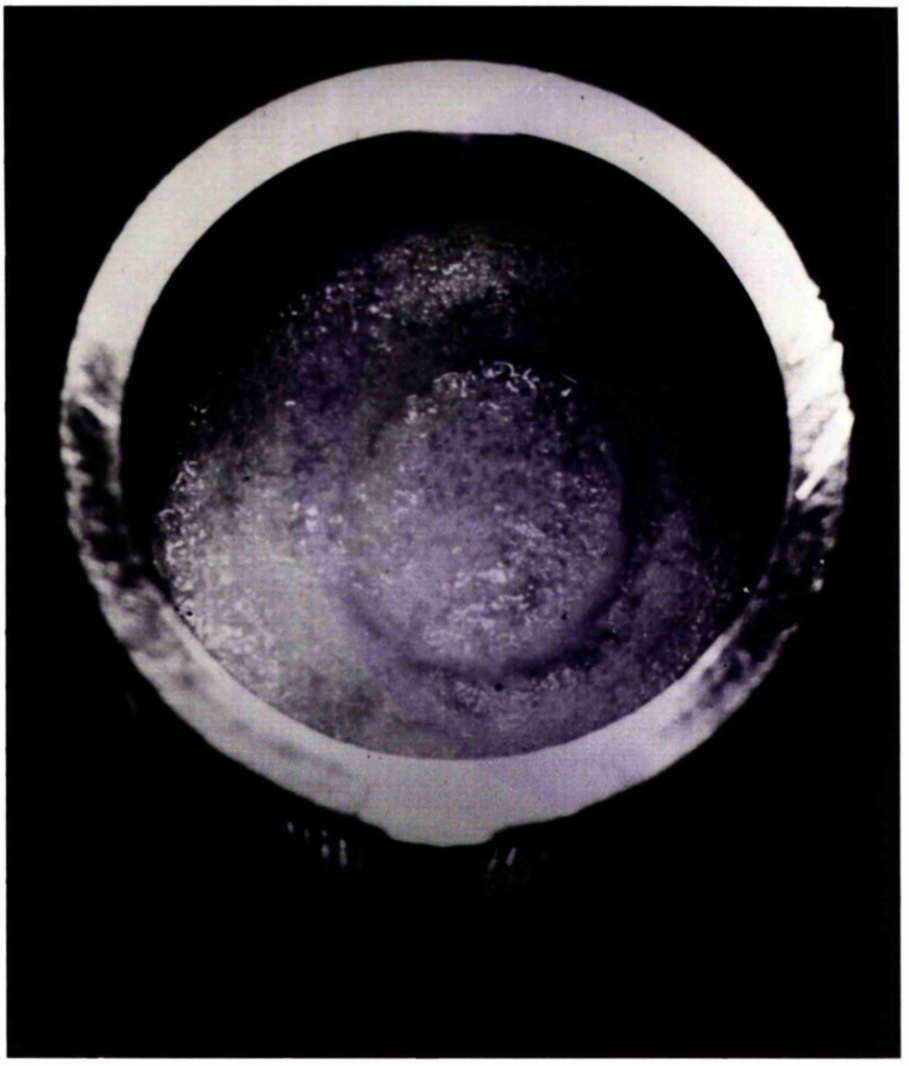

Figure 23 Appearance of a capsule in the basket at the end of the dissolution rate test.

digoxin solution while tablets with digoxin particles of 102μm gave less than half this absorption. Dissolution rate increased as particle size reduced. Beveridge et al., (1975) noted reduced absorption from capsules containing "coarse particles" of digoxin. In the study of Johnson, O'Grady and Bye (1978) cited earlier the four day urinary digoxin excretion after single doses with tablets of large particle digoxin was just under half that after standard Lanoxin.

The log-normal distribution of digoxin particle diameters is consistent with the shape of the curves obtained in tablet dissolution rate tests (Brooke 1978), which show the rate of release into solution progressively falling over several hours (Fraser et al., 1974). The 50% of the particles in the lower end of the log-normal size distribution are grouped into a narrower range of particle diameters and, in addition, since volume and mass increase with cube of the radius a greater proportion of the dose is contained in a single large particle than in a single small particle.

The finding that the digoxin powders used by British digoxin tablet manufacturers had a relatively large particle size which gave poor digoxin absorption suggests that the milling processes in production were critical in determining the bioavailability of the final product.

Particle size had been known in the 1960's to be an important determinant for dissolution rate and absorption of several poorly soluble drugs, such as griseofulvin and chloramphenicol. In view of the promptings which had been given by academic pharmacists such as Levy and Nelson (1961) and Wagner (1971) it is disappointing that

it was only after the discovery of variable digoxin absorption in clinical studies that the pharmaceutical companies became aware of the digoxin bioavailability problem.

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